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REPORT P 0268/2179

Mutagenicity of the Mainstream and Sidestream Whole Smoke Condensates of the Research Cigarettes CALYPSO-1, AREUSE-46, -53, and -55 in the Salmonella Typhimurium Strains TA98 and TA100

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Sitz der Gesellschaft: Köln HR B 367
Geschäftsführer: Dr. Wolf Reinghaus

Erratum

Re.: Final Report P 0268/2179, Project AREUSE

After the delivery of the Final Report P 0268/2179, dated 23 Nov.90, the client gave us the information in Feb. 1991 that the humectant concentration data were not correct. Therefore, the following data in TABLE 1 on PAGE 20 have to be replaced (*):

Cigarette	Humectant Conc. in the Filler (%)		
	PG	GLY	TEG
CALYPSO-1	1.5	2.5	-
AREUSE-46	"	"	-
-53	"	"	1.0
-55	"	7.5	

Remarks: PG: propylene glycol
GLY: glycerol
TEG: triethylene glycol

10. Feb. 1995

*) from: Reininghaus W., Speck M.: On the influence of humectants on..., Summary Report P 0268/2160, dated 22 Mar.91

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ABBREVIATIONS (a)(b)
==========

2-AA : 2-aminoanthracene
2-AF : 2-aminofluorene
AHM : aryl hydrocarbon monooxygenase (EC 1.14.14.2)
CFU : colony-forming unit
DIN : Deutsches Institut für Normung (German Committee of Standards)
DMSO : dimethyl sulfoxide
EC : enzyme code according to the "International Union of Biochemistry, Commission on Enzymes"
Ex : x as exponent to the base 10, e.g, E2 = 10^2
FID : flame ionization detector
x g : centrifugal force in terms of the constant of gravitation (1 x g = 9.81 m/s2)
G6P : glucose-6-phosphate
M : arithmetic mean
MMS : methyl methanesulfonate
MWSC-I: mainstream whole smoke condensate collected with impaction trap
N : number of individual values
NADP : nicotinamide adenine dinucleotide phosphate
NADPH : nicotinamide adenine dinucleotide phosphate, reduced form
PBS : phosphate-buffered saline
PT : preliminary title
PTFE : polytetrafluorethylene
r : correlation coefficient
rpm : revolutions per minute
RSD : relative standard deviation
RT : room temperature
S9 : supernatant of 9000 x g centrifugation

(a) in addition to those explained immediately on the same page
(b) Units are given in accordance with SI units (Système International d'Unités).

SE : standard error
SOP : standard operating procedure
SWSC-I: sidestream whole smoke condensate collected with impaction trap
U : unit
vs : versus
WSC-I : whole smoke condensate collected with impaction trap

1 SUMMARY

1.1 Objective

Within the framework of the FTR project AREUSE, this study was designed to investigate the mutagenicity of the MAINSTREAM and SIDESTREAM WHOLE SMOKE CONDENSATES of the research cigarettes CALYPSO-1, AREUSE-46, -53, and -55. The mutagenicity determination was carried out using the Salmonella typhimurium reverse mutation assay with the tester strains TA98 and TA100 in the presence of a metabolic promutagen activation system.

1.2 Cigarettes

CALYPSO-1 contained the original blend with 2.1 percent propylene glycol and 3.0 percent glycerol as humectants in the filler. AREUSE-46 had the same humectant concentrations but the blend was modified by replacing stems with an additional amount of flue-cured tobacco. It was the reference cigarette for AREUSE-53 and -55. These cigarettes differed from the reference cigarette in that AREUSE-53 contained 1 percent triethylene glycol as an additional humectant and that AREUSE-55 had a glycerol concentration in the filler of 8 percent.

1.3 Experimental Conduct

The mainstream and sidestream whole smoke was simultaneously generated using automatic INBIFO smoking machines. The condensates were collected with glass impaction traps (MWSC-I, SWSC-I). Four batches of each type of condensate per research cigarette were

prepared and assayed at the doses 0, 0.05, 0.10, and 0.15 milligrams dry condensate per plate.

The mutagenicity assay performed was a plate incorporation assay. Salmonella typhimurium strains TA98 and TA100 were used to detect mutagens which induce frameshift mutation and base-pair substitution respectively. For each tester strain, 2 independent consecutive substudies were carried out using 2 individual condensate batches and 4 plates per dose in each substudy. A postmitochondrial (S9) fraction from the livers of rats treated with Aroclor 1254 was used for the metabolic promutagen activation of the whole smoke condensates. Mutation events were detected in tester strain bacteria reverted from histidine auxotrophy to prototrophy by growth on histidine-deficient agar plates. The number of revertants at the given doses was used to calculate the linear dose-response curve. From this curve, the mutagenicity was calculated as the increase in the number of revertants per milligram dry condensate (specific mutagenicity). Further, the mutagenicity of the condensates was calculated per milligram "new tar", i.e., dry condensate minus nicotine minus humectant content.

The assays were carried out according to the OECD guideline no. 471 (1983). However, only strains TA98 and TA100 were used. These tester strains were found to be in accordance with their genotypes, their spontaneous reversion, and their response to diagnostic mutagens.

1.4 Results

1.4.1 Humectant concentration in condensate

Propylene glycol could not be determined because it could not be separated from the solvent DMSO. The glycerol yield of the MWSC-I

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was approx. 1.3 milligrams per cigarette for CALYPSO-1, AREUSE-46, and -53 and 3.6 milligrams per cigarette for AREUSE-55. For the SWSC-I, it was approx. 1.8 and 6.3 milligrams per cigarette. The triethylene glycol yield of the MWSC-I was <0.1 milligrams per cigarette for CALYPSO-1, AREUSE-46, and -55 and 1.0 milligram per cigarette for AREUSE-53. For the SWSC-I, it was ≤0.1 and 1.2. These humectant yields correlated with the concentrations determined in the filler.

1.4.2 Dry condensate yield

The dry condensate yield of the MWSC-I of CALYPSO-1, AREUSE-46, -53, and -55 was approx. 17 milligrams per cigarette. For the SWSC-I, it was 22, 23, 25, and 28 milligrams per cigarette respectively.

1.4.3 Mutagenicity

The mutagenic activity of the MWSC-I and SWSC-I of the research cigarettes found in substudy 1 was reproduced in substudy 2. The specific mutagenicity was as follows:

TYPE OF CONDENSATE	TESTER STRAIN, MUTATION	CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg dry condensate) M (b)	SE	RELATIVE DIFFERENCE (a) TO CALYPSO-1	AREUSE-46
MWSC-I	TA98, frameshift mutation	CALYPSO-1	2180	64	-	-0.10=
		AREUSE-46	2401	95	0.10=	-
		-53	2317	92	0.06=	-0.04=
		-55	2119	61	-0.03=	-0.12=
	TA100, base-pair substitution	CALYPSO-1	984	85	-	-0.03=
		AREUSE-46	1009	61	0.03=	-
		-53	955	31	-0.03=	-0.05=
		-55	867	48	-0.13=	-0.15=
SWSC-I	TA98, frameshift mutation	CALYPSO-1	1781	82	-	-0.03=
		AREUSE-46	1827	81	0.03=	-
		-53	1546	74	-0.14=	-0.17+
		-55	1282	52	-0.33+	-0.35+
	TA100, base-pair substitution	CALYPSO-1	1130	52	-	0.02=
		AREUSE-46	1113	35	-0.02=	-
		-53	991	40	-0.13=	-0.12=
		-55	880	28	-0.25+	-0.23+

Only relative differences ≥0.10 between the research cigarettes are discussed.

The specific mutagenicity of the MWSC-I of the test cigarette AREUSE-55 was numerically lower than that of the reference cigarette AREUSE-46 with respect to both types of mutation. MWSC-I of CALYPSO-1 was less mutagenic than that of AREUSE-46 with

(a) difference between the specific mutagenicities of the test cigarette and the cigarettes CALYPSO-1 or AREUSE-46 divided by the mean of them, level of significance set at alpha = 0.05, reached at a relative difference between 2 research cigarettes >0.16 (absolute value)

=: no statistically significant difference
+: statistically significant difference

(b) N = 4 condensate batches

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respect to frameshift mutation and more mutagenic than that of AREUSE-55 with respect to base-pair substitution. However, none of these differences were statistically significant.

The specific mutagenicity of the SWSC-I of the test cigarettes AREUSE-53 and -55 was lower than that of the reference cigarette AREUSE-46 with respect to both types of mutation. The differences were statistically significant in 3 of 4 cases. SWSC-I of CALYPSO-1 was more mutagenic than that of AREUSE-53 and -55 with respect to both types of mutation, a statistical significance being observed for AREUSE-55.

1.5 Comment

The modification of the original blend by replacing stems with an additional amount of flue-cured tobacco was found to slightly increase the specific mutagenicity of the MWSC-I (CALYPSO-1 vs AREUSE-46).

Increasing triethylene glycol from 0 to 1 percent and glycerol from 3 to 8 percent in the modified blend were found to decrease the mutagenicity on a dry condensate basis (AREUSE-46 vs -53 and -55). However, when the mutagenicity was calculated on a "new tar" basis, the differences between the reference and the test cigarettes were no longer seen. Therefore, the reduction in the mutagenicity on a dry condensate basis seems to be related to a dilution effect caused by the transferred humectants.

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Institut für biologische
Forschung GmbH

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2 RESPONSIBILITY

2.1 Project Management

Study Director: 27.Nov.90 ..... Dr. Tewes
Date Dr.rer.nat. F. Tewes
Biologist (Diplombiologe)

2.2 Contributing Teams

Analytical Chemistry: 27.Nov.90 ..... P. Voncken
Date Dr.rer.nat. P. Voncken
Chemist (Diplomchemiker)

Microbiology: 27.Nov.90 ..... Dr. Tewes
Date Dr.rer.nat. F. Tewes
Biologist (Diplombiologe)

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3 QUALITY ASSURANCE STATEMENT

The study was conducted according to the Good Laboratory Practice Regulations (a).

Inspections on this study were performed by the quality assurance unit on 11, 15, and 16 Aug.89. All findings were immediately reported to the study director and to the general management.

This report accurately reflects the study carried out and the results obtained.

29. Nov. 1990

....

Date Dr.med. U. Hackenberg
Pharmacologist and Toxicologist
(Pharmakologe und Toxikologe)

(a) Federal Register (1987)

4 RESEARCH SUBSTANCES

4.1 General Specification

Research substance:	mainstream and sidestream whole smoke condensate of 4 research cigarettes (a) collected with glass impaction traps blend composition and humectant concentration: see TABLE 1
Research cigarettes	
Code:	(1) CALYPSO-1 (2) AREUSE-46 (3) AREUSE-53 (4) AREUSE-55
Source:	FTR
Date of receipt at INBIFO:	15 Jun.89
INBIFO substance no.:	(1) Z 1135A (2) Z 1136A (3) Z 1137A (4) Z 1138A
Labeling on cigarette pack:	(1) CALYPSO 001 P (2) AREUSE 046 P (3) AREUSE 053 P (4) AREUSE 055 P
Amount:	1200
Packaging:	approx. 300 single cigarettes/plastic box
Storage:	in walk-in cold room (R922), approx. 4 °C, relative humidity uncontrolled

(a) In addition to the research cigarettes, the standard reference cigarette 2R1 was used as internal control.

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	prior to conditioning cigarettes stored at approx. -20 °C for approx. 24 h and subsequently equilibrated at RT before opening
Conditioning:	in conditioning room (R326) for approx. 7 d prior to use at approx. 23 °C, 60 % relative humidity
	cigarettes taken out of their boxes and deposited in approx. 7 horizontal layers
Selection:	no selection

Condensate

Preparation:	see Chapter 5.2
Number of condensate batches:	4 condensate batches/condensate type
Number of single cigarettes per condensate batch:	approx. 240
Solvent:	DMSO
Specification:	yield of dry condensate, water, and nicotine and puff count determined for each WSC-I batch
Storage:	in the dark at 4 °C, approx. 7 d prior to the mutagenicity assay

Scientific version:	SOP MB 84/2, QA 8/7
Text version:	21 Apr.89

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4.2 Supplier's Specification

CIGARETTE	BLEND COMPOSITION (%)						HUMECTANT CONC. IN THE FILLER (%) (a)		
	BU	FC	OR	RL	ET	IS	PG	GLY	TEG
CALYPSO-1	27.2	15.7	15.0	24.8	14.0	3.3	2.1	3.0	-
AREUSE-46	27.9	18.8	15.0	24.3	14.0	0	"	"	-
-53	"	"	"	"	"	"	"	"	1.0
-55	"	"	"	"	"	"	"	8.0	-

TABLE 1 CIGARETTE BLEND COMPOSITION AND HUMECTANT CONCENTRATION

Remarks: BU: Burley tobacco
FC: flue-cured tobacco
OR: oriental tobacco
RL: reconstituted leaf
ET: expanded tobacco
IS: improved stems
PG: propylene glycol
GLY: glycerol
TEG: triethylene glycol

(a) The concentrations of GLY in cigarette AREUSE-53 and TEG in AREUSE-55 included in the supplier's analysis data sheets are probably not correct (see also Chapter 7.1.1, and TABLES 5 to 7)

S.a. Tab. 5 Seite 81

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Wrapper: WP60

1 33

3303TE001P 02896027

33.120 DATE 14/02/89 TIME 08.22

ANALYSIS PROJECT LEADER SAP
PMI SMOKING
LABORATORY PROJECT NR. 4027 PROTOTYPE NR. 001P

TYPE OF ANALYSIS BAR CODE FENG783

TEST DATE O.ANALYSIS 02/89

CIGARETTE					
CIG. LENGTH, TOTAL	MM 21	085	CIG. WEIGHT, TOTAL	MG/CIG 29	1068
BUTT LENGTH	MM 22	029	F+P WT	MG/CIG 30	218
SMOKED LENGTH	MM 23	056	TOB WT	MG/CIG 31	0790
ROD LENGTH CIGARETTE	MM 24	064	CIG. DIAMETER	MM 25	7.93
ROD (TOBACCO) VOLUME	ML 26	3.163	CIG. RTD	MM H2O 33	104
BURN.TIME MIN/40 MM	35		CIG. VENTILATION	% 58	15
PAP POR ML/CM2.MIN	27		FIRMNESS AT 12.5 % OV	MM 34	3.30
C(. PAP. VENTIL. SYST.	28		EXPANDED TOBACCO	% 32	
FILLING POWER CV ML/10G	07				
FIRMNESS AS IS	MM 08	3.06			
OVEN VOLATILES AS IS	% 09	11.9			
FILLER					
ALKALOIDS, TOTAL	% 74	1.79	KJELDAHL NITROGEN	% 78	
REDUCING SUGARS	% 75	07.5	NITROGEN, TOTAL	% 79	
NITRATE NITROGEN	% 76	0.22	OV AT EQUILIBRIUM	% 71	14.7
AMMONIA NITROGEN	% 77	0.31	WEIG 12.5	MG/CIG 72	0770
			DENSITY	MG/ML 73	244
FILTER					
FILTER LENGTH,TOTAL	MM 51	21	FILTER RTD	MM H2O 57	072
FILTER-LENGTH PLUG 1	MM 53		FILTER TYPE	59	S
FILTER-LENGTH PLUG 2	MM 52		FM FILTER MATERIAL	60	CA
FILTER-LENGTH PLUG 3	MM 41		DEN.SIN1 68	SECT.- 69	
TIPPING LENGTH ON CIG.	MM 54	25	DEN.SIN2 61	SECT.- 62	
TIPPING PAPER TYPE	55	IC	DEN.SIN3 39	SECT.- 60	
T( )ING PEREO. TYPE	18	EP7	FA FIL.ADDITIVE TYPE	63	
TIPPING,PEREO. LINES NO.	65		FA FIL.ADDITIVE CONTMG/FIL	64	
FILTER WEIGHT MG/FIL	56	167			
TPM FR MG/CIG	50				
WATER FR MG/CIG	69				
DPM FR MG/CIG	65				
SN F MG/FIL	66				
FILTER EFFICIENCY %	67				
SMOKE					
CO CARBON MONOXIDE MG/CIG	81	17.4	NO NITROGEN MONOXIDEMG/CIG	82	0.26

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
TPM	MG/CIG 83	18.0	18.1	18.7	18.5	18.4	17.5	17.8	19.2	= 18.3
H2O WATER IN TPM	MG/CIG 80	2.2	2.3	2.5	2.5	2.4	1.9	2.4	2.8	= 2.4
DPM	MG/CIG 86	15.9	15.8	16.2	16.1	16.0	15.6	15.4	16.5	= 15.9
TAR	MG/CIG 90		14.9	15.2	15.1	15.0	14.8	14.5	15.5	= 15.0
SN SMOKE NICOTINE	MG/CIG 85		0.95	0.97	0.94	0.97	0.89	0.92	0.98	= 0.95
RATIO SN/TAR	% 10									
PUFF COUNT	NO./CIG 86	8.6	8.4	8.8	8.8	9.1	8.6	8.4	9.2	= 8.7
HCN	UG/CIG 88									
ALDEHYDES, TOTAL	MG/CIG 89									

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3303TE246P 0389?611

33,120 DATE 28/03/89 TIME 06.33

ANALYSIS PROJECT LEADER SPM

PME SMOKING

LABORATORY PROJECT NR. 0611 PROTOTYPE NR. 046P

TYPE OF ANALYSIS BAR CODE FEN0542

ML mod (1)

TEST DATE O.ANALYSIS 03/89

CIGARETTE							
CIG. LENGTH, TOTAL	MM	21	084	CIG. WEIGHT, TOTAL	MG/CIG	29	1016
BUTT LENGTH	MM	22	029	F+P WT	MG/CIG	30	220
SMOKED LENGTH	MM	23	055	TOB WT	MG/CIG	31	0796
ROD LENGTH CIGARETTE	MM	24	063	CIG. DIAMETER	MM	25	7.92
ROD (TOBACCO) VOLUME	ML	26	3.107	CIG. RTD	MM H20	33	105
BURN.TIME 9IN/4C	MM	35		CIG. VENTILATION	%	58	14
PAP POR 9L/CM2.MIN		27		FIRMNESS AT 12.5 + OV	MM	34	2.89
CIG. PAP. VENTIL. SYST.		28		EXPANDED TOBACCO	%	32	
FILLING POWER CV 9L/10G		07					
F. MNESS AS IS	MM	08	2.70				
OVEN VOLATILES AS IS	%	09	12.0				
FILLER							
ALKALOIDS, TOTAL	%	74	1.98	KJELDAHL NITROGEN	%	78	
REDUCING SUGARS	%	75	07.7	NITROGEN, TOTAL	%	79	
NITRATE NITROGEN	%	76	C.18	OV AT EQUILIBRIUM	%	71	13.0
AMMONIA NITROGEN	%	77	0.29	WEIG 13.5	MG/CIG	72	0791
				DENSITY	MG/ML	73	255
FILTER							
FILTER LENGTH,TOTAL	MM	51	21	FILTER RTD	MM H20	57	075
FILTER-LENGTH PLUG 1	MM	53		FILTER TYPE		59	S
FILTER-LENGTH PLUG 2	MM	52		FM FILTER MATERIAL		60	CA
FILTER-LENGTH PLUG 3	MM	61		DEN.SIN1 68	SECT.-	69	
TIPPING LENGTH ON CIG.	MM	54	25	DEN.SIN2 61	SECT.-	62	
TIPPING PAPER TYPE		55	IC	DEN.SIN3 39	SECT.-	40	
TIPPING PERFO. TYPE		18	EPZ	FA FIL.ADDITIVE TYPE		63	
TIPPING PERFO. LINES NO.		65		FA FIL.ADDITIVE CONTMG/FIL		64	
F. ER WEIGHT	MG/FIL	56	170				
TPM FR	MG/CIG	50					
WATER FR	MG/CIG	49					
DPM FR	MG/CIG	65					
SN F	MG/FIL	66					
FILTER EFFICIENCY	%	67					
SMOKE							
CO CARBON MONOXIDE	MG/CIG	81	16.6	NO NITROGEN MONOXIDEMG/CIG	82	0.24	

			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
TPM	MG/CIG	83	21.2	21.3	20.6	21.5	21.8	21.7		20.9 =	21.3
H2O WATER IN TPM	MG/CIG	80	3.0	2.6	2.7	2.6	2.6	2.8		2.6 =	2.7
DPM	MG/CIG	84	18.2	18.7	17.9	19.0	19.3	18.9		18.3 =	18.6
TAR	MG/CIG	90	17.0	17.4	16.7	17.7	18.0	17.6		17.0 =	17.3
SM SMOKE NICOTINE	MG/CIG	85	1.25	1.31	1.24	1.33	1.29	1.31	1.27	1.28 =	1.29
RATIO SM/TAR	%	10									7.4
PUFF COUNT	NO./CIG	86	9.7	10.3	9.8	10.3	10.3	9.5	9.9	9.6 =	9.9
M C N	UG/CIG	88									
ALDEHYDES, TOTAL	MG/CIG	89									

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3303TE353P C4390611

33.120 DATE 21/04/89 TIME 07.2

ANALYSIS PROJECT LEADER SPM

PME SMOKING

LABORATORY PROJECT NR. 0611 PROTOTYPE NR. 353P

TYPE OF ANALYSIS BAR CODE FEA[illegible] ML + 5% Gly

TEST DATE O.ANALYSIS C4/89

CIGARETTE							
CIG. LENGTH, TOTAL	MM	21	C84	CIG. WEIGHT, TOTAL	MG/CIG	29	1007
BUTT LENGTH	MM	22	029	FAP WT	MG/CIG	30	214
SMOKED LENGTH	MM	23	055	TOB WT	MG/CIG	31	0793
ROD LENGTH CIGARETTE	MM	24	C63	CIG. DIAMETER	MM	25	7,91
ROD (TOBACCO) VOLUME	ML	26	3,097	CIG. RTD	MM H2O	33	096
BURN.TIME MIN/4C	MM	35		CIG. VENTILATION	%	55	19
PAP POR ML/C42.MIN		27		FIRMNESS AT 12.5 - OV	MM	34	2,77
CIG. PAP. VENTIL. SYST.		28		EXPANDED TOBACCO	%	32	
FILLING POWER CV ML/10G		37					
FIRMNESS AS IS	MM	38	2,79				
OVEN VOLATILES AS IS	%	39	12,6				
FILLER							
ALKALOIDS, TOTAL	%	74	1,96	KJELDAHL NITROGEN	%	78	
REDUCING SUGARS	%	75	08,6	NITROGEN, TOTAL	%	79	
NITRATE NITROGEN	%	76	C,17	OV AT EQUILIBRIUM	%	71	13,7
AMMONIA NITROGEN	%	77	C,33	WEIG 12.5	MG/CIG	72	0782
				DENSITY	MG/ML	73	253
FILTER							
FILTER LENGTH,TOTAL	MM	51	21	FILTER RTD	MM H2O	57	069
FILTER-LENGTH PLUG 1	MM	53		FILTER TYPE		59	S
FILTER-LENGTH PLUG 2	MM	52		FM FILTER MATERIAL		60	CA
FILTER-LENGTH PLUG 3	MM	61		DEN.SIN1 58	SECT.-	69	
TIPPING LENGTH ON CIG.	MM	54	25	DEN.SIN2 61	SECT.-	62	
TIPPING PAPER TYPE		55	ICD	DEN.SIN3 39	SECT.-	40	
TIPPING PERFO. TYPE		18	EP2	FA FIL.ADDITIVE TYPE		63	
TIPPING,PERFO. LINES	NO.	65		FA FIL.ADDITIVE CONT	MG/FIL	64	
FILTER WEIGHT	MG/FIL	56	166				
TPM FR	MG/CIG	50					
WATER FR	MG/CIG	69					
DPM FR	MG/CIG	65					
SM F	MG/FIL	66					
FILTER EFFICIENCY	%	67					
SMOKE							
CO CARBON MONOXIDE	MG/CIG	81	15,2	NO NITROGEN MONOXIDE	MG/CIG	82	0,23

			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
TPM	MG/CIG	83	20,8	19,7	20,7	19,4	21,4	19,5	21,4	19,5	= 20,3
H2O WATER IN TPM	MG/CIG	80	2,2	2,5	2,6	2,3	2,5	2,3	2,7	2,3	= 2,6
DPM	MG/CIG	86	18,6	17,2	18,2	17,1	19,C	17,2	18,9	17,2	= 17,9
TAR	MG/CIG	90	17,3	16,1	17,0	16,0	17,8	16,1	17,7	16,0	= 16,8
SM SMOKE NICOTINE	MG/CIG	85	1,24	1,12	1,22	1,13	1,21	1,13	1,19	1,13	= 1,17
RATIO SM/TAR	%	10									7,0
PUFF COUNT	NO./CIG	96	9,9	9,5	9,7	9,2	9,6	9,C	9,9	9,3	= 9,5
N C N	UG/CIG	88									
ALDEHYDES, TOTAL	MG/CIG	89									

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1 33
3303TE055P 04890611

33.120 DATE 21/04/89 TIME 07.25

ANALYSIS PROJECT LEADER SPM
PME SMOKING
LABORATORY PROJECT NR. 0611 PROTOTYPE NR. 055P

TYPE OF ANALYSIS BAR CODE FENC532

TEST DATE O.ANALYSIS 04/89

CIGARETTE							
CIG. LENGTH, TOTAL	MM 21	084	CIG. WEIGHT, TOTAL	MG/CIG	29	1013	
BUTT LENGTH	MM 22	029	F+P WT	MG/CIG	30	219	
SMOKED LENGTH	MM 23	055	TOB WT	MG/CIG	31	0794	
ROD LENGTH CIGARETTE	MM 24	063	CIG. DIAMETER	MM	25	7.92	
ROD (TOBACCO) VOLUME	ML 26	3.103	CIG. RTD	MM H2O	33	088	
BURN.TIME MIN/40	MM 35		CIG. VENTILATION	%	58	23	
PAP POR ML/CM2.MIN	27		FIRMNESS AT 12.5 . DV	MM	34	3.47	
CIG. PAP. VENTIL. SYST.	28		EXPANDED TOBACCO	%	32		
FILLING POWER CV ML/10G	37						
THICKNESS AS IS	MM 38	4.09					
OVEN VOLATILES AS IS	% 39	14.1					
FILLER							
ALKALOIDS, TOTAL	% 74	1.88	KJELDAHL NITROGEN	%	78		
REDUCING SUGARS	% 75	08.9	NITROGEN, TOTAL	%	79		
NITRATE NITROGEN	% 76	0.18	OV AT EQUILIBRIUM	%	71	15.6	
AMMONIA NITROGEN	% 77	0.34	WEIG 12.5	MG/CIG	72	0765	
			DENSITY	MG/ML	73	247	
FILTER							
FILTER LENGTH,TOTAL	MM 51	21	FILTER RTD	MM H2O	57	069	
FILTER-LENGTH PLUG 1	MM 53		FILTER TYPE		59	S	
FILTER-LENGTH PLUG 2	MM 52		FM FILTER MATERIAL		60	CA	
FILTER-LENGTH PLUG 3	MM 61		DEN.SIN1 68	SECT.-	69		
TIPPING LENGTH ON CIG.	MM 54	25	DEN.SIN2 61	SECT.-	62		
TIPPING PAPER TYPE	55	10	DEN.SIN3 39	SECT.-	40		
TIPPING PERFO. TYPE	18	EP2	FA FIL.ADDITIVE TYPE		63		
TIPPING PERFO. LINES NO.	45		FA FIL.ADDITIVE CONTMG/FIL		64		
F.TEP WEIGHT MG/FIL	56	168					
TPM FR MG/CIG	50						
WATER FR MG/CIG	69						
DPM FR MG/CIG	65						
SN F MG/FIL	66						
FILTER EFFICIENCY %	67						
SMOKE							
CO CARBON MONOXIDE MG/CIG	81	15.4	NO NITROGEN MONOXIDEMG/CIG	82	1.21		

			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	
TPM	MG/CIG	83	22.8	21.6	22.9	21.7	20.7	20.4	20.3	21.1	= 21.3
H2O WATER IN TPM	MG/CIG	80	3.0	3.2	3.3	2.7	3.0	2.7	3.2	2.8	= 3.0
DPM	MG/CIG	84	17.8	18.4	17.6	19.0	17.7	17.7	17.6	18.3	= 18.0
TAR	MG/CIG	30	16.7	17.3	16.5	17.8	16.7	16.6	16.6	17.2	= 16.9
SN SMOKE NICOTINE	MG/CIG	85	1.08	1.17	1.08	1.13	1.05	1.06	1.04	1.10	= 1.08
RATIO SN/TAR	%	10									6.4
PUFF COUNT	NO./CIG	86	9.1	10.0	9.5	10.1	9.3	9.4	9.6	9.9	= 9.7
NCM	UG/CIG	88									
ALDEHYDES, TOTAL	MG/CIG	89									

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5 METHOD

5.1 Chronology

(see FIGURE 1)

5.2 Condensate Preparation, Suspension, Storage, and Analyses

5.2.1 Mainstream and sidestream whole smoke condensate preparation

Principle:	mechanical open-end smoking to a defined butt length in an automatic negative pressure (vacuum pump) smoking machine mainstream smoke collection with impaction trap sidestream smoke collection by means of a circular hood and a special impaction trap
Time:	see FIGURE 1
Sample material and quantity:	cigarettes, approx. 240/batch

Equipment

Smoking machine

Type:	30-port automatic INBIFO smoking machine with a circular hood for sidestream smoke collection
Number of machines:	2
Machine no.:	0035, 0036
Loading of cigarettes:	automatically into the cigarette holding device up to a depth of 9 ± 1 mm in accordance with DIN 10240

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Lighting of cigarettes:	automatically or manually with an iodine spot lamp iodine spot lamp: Halogen-Bellaphot, 15 V, 150 W, gold-plated reflector, Osram, no. 64635, R. Schahl, D-8000 München 71
Ejection of cigarettes:	automatically at butt length of at least 23 mm, but not less than the length of the filter + 8 mm or not less than the length of the tipping paper + 3 mm, in accordance with DIN 10240

Vacuum pump

Mainstream smoke:	membrane vacuum pump N 0135/AVE, K. Neuberger KG, D-7800 Freiburg-Munzingen
Sidestream smoke:	water ringpump LRKA 10603, SIHI Halberg, via Hartmann und Essen GmbH und Co., D-5000 Köln 91
Flowmeter:	rotameter, L 4/160, Rota, Dr. Henning KG, D-7867 Wehr/Baden soap-film flowmeter, Faust GmbH, D-5000 Köln 90

Impaction trap for mainstream smoke

Type:	glass "impaction trap for cigarette smoke condensate collection" according to Philip Morris (see FIGURE 2), Faust GmbH, D-5000 Köln 90
Capillary:	length: 5 mm bore: 0.4 mm

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Mode of installation of the impaction trap insert:	distance of 0.5 mm between capillary tip and wall of flask calibrated with a PTFE sheet spacer with a thickness of 0.5 mm
Connection of impaction trap of smoking machine:	impaction trap lies horizontally below smoking machine connected via glass tube
	dimension of connecting glass tubes (between impaction trap and smoking machine): length: 51 cm outer diameter: 13 mm inner diameter: 8 mm

Impaction trap for sidestream smoke

Type:	glass impaction trap for sidestream smoke condensate collection (see FIGURE 3), Faust GmbH, D-5000 Köln 90
Outlet nozzle:	annular fissure, 88 mm length and 0.1 mm width
Distance of the impaction plate from the outlet nozzle:	approx. 0.1 mm
Installation of impaction trap:	in vertical position in an ice/water bath below smoking machine connected via copper tube with sidestream smoke collection hood
	dimension of copper tube: length: 70 cm outer diameter: 35 mm inner diameter: 30 mm

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Procedure

Puff frequency/cigarette:	1 puff/min
Puff duration:	2 s
Puff volume:	35 ml
Puff profile:	rectangular
Suction flow rate	
Mainstream smoke:	1.05 l/min
Sidestream smoke:	approx. 120 l/min, range: 90 to 160 l/min
Pressure in impaction trap	
Mainstream smoke:	approx. 4E4 Pa (0.4 bar)
Sidestream smoke:	approx. 6E4 Pa (0.6 bar), regulated with pressure gauge
Scientific version:	SOP AC 41/2, AC 42/2
Text version:	23 Aug.89

5.2.2 Suspension and storage of condensate

Principle:	suspension of WSC-I in DMSO by sonication
Time:	immediately after WSC-I preparation
Sample material and quantity:	total WSC-I of each condensate batch
Results expressed in:	g/l, mg/cigarette

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Equipment:	sonication water bath: Sonorex RK 100, Bandelin KG, D-1000 Berlin
	brown glass bottles, 125 ml, no. 219885, with PTFE-lined screw caps, brown glass vials, 8 ml, no. 224984, screw caps, no. 240409, Wheaton Scientific, via Zinsser, D-6000 Frankfurt/Main
	brown glass bottles, 100 ml, no. 704046 (screw caps from Wheaton Scientific, see above), Brand GmbH und Co., via Faust GmbH, D-5000 Köln 90
Chemicals:	DMSO, no. 2950, E. Merck, D-6100 Darmstadt 1
Procedure:	WSC-I washed out of trap approx. 8 times with approx. 10-ml portions of DMSO repeatedly after sonication (water bath) for approx. 5 min, washings transferred to a 100-ml volumetric flask and filled up to volume with DMSO
	amount of WSC-I calculated from weight of impaction trap before and immediately after condensate preparation
	concentration of dry condensate calculated from WSC-I and water concentration of suspension (determination of water concentration: see Chapter 5.6.1)

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Storage:	in sterile brown glass bottles at 4 °C for approx. 7 d, 5-ml aliquots at -75 °C
	labeling of the bottles: study no., batch no., condensate type, cigarette short code, date of condensate preparation
Scientific version:	SOP AC 52/1, AC 74/2
Text version:	22 Mar.88

5.2.3 Analyses

5.2.3.1 Determination of water concentration

Principle:	titration according to Karl Fischer modified by E. Scholz (1984)
Time:	within 48 h after preparation of WSC-I suspension
Sample material and quantity:	WSC-I/DMSO suspension, 0.5 ml, 2 determinations/suspension
Results expressed in:	g/l and mg/cigarette
Equipment:	Karl Fischer-Titrator DL18, Printer GA44, Mettler Waagen GmbH, D-6300 Giessen
Chemicals:	Hydranal composite 5, no. 34805, Hydranal solvent, no. 34800, Hydranal-Eichstandard 5.00, no. 34813, Riedel-de Haen, D-3016 Seelze 1

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DMSO, no. 2950,
E. Merck,
D-6100 Darmstadt

Procedure

Calibration:	20 ml Hydranal solvent plus 0.5 ml DMSO titrated with composite 5 solution to dryness, stop time 15 s, 0.5 ml "Eichstandard 5.00" titrated in instrument mode for calibration, calibration factor stored automatically, 0.5 ml DMSO titrated, blank value stored in configuration file
Titration:	20 ml Hydranal solvent titrated to dryness, stop time 15 s, for titration of the sample the method "fliegender Start" used, e.g., start of the titration and within 15 s addition of 0.5 ml of the sample
Computation:	result automatically printed after titration is finished
Reproducibility (RSD):	4 % (determined at 10 g/l, N = 5)

Scientific version: SOP AC 16/4
Text version: 18 Jul.88

5.2.3.2 Determination of nicotine concentration

Principle:	dilution of condensate suspension in DMSO with n-butylacetate containing isoquinoline as internal standard, capillary gas chromatographic determination of nicotine, data acquisition and evaluation using a laboratory data system

Time:	within 48 h after preparation of WSC-I suspension
Sample material and quantity:	condensate suspension in DMSO, 1 ml
Results expressed in:	g/l, mg/cigarette
Equipment:	gas chromatograph: HP5890 with autosampler HP 7673A, Hewlett-Packard GmbH, D-4030 Ratingen
	laboratory data system:
	hardware: Microvax II, VT340, LA 210, Digital Equipment GmbH, D-8000 München
	software: Multichrom, VG Instruments GmbH, D-6200 Wiesbaden
	centrifuge: model J-6 B, rotor: JS-4.2, Beckman Instruments GmbH, D-8000 München 40
	capillary column: J and W, DB5, no. 123-5025, Carlo Erba Instruments, D-6238 Hofheim
	digital dispensette 2 to 10 ml, Eppendorf pipette 1 ml, centrifuge tubes, 20 ml with PTFE-lined septum and screw cap, Faust GmbH, D-5000 Köln 90
Chemicals:	n-butylacetate, no. 27,068-7, triethylamine, no. 23,962-3, Aldrich Chemie GmbH und Co., D-7924 Steinheim

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nicotine, no. 77635,
Serva Feinbiochemica GmbH und Co.,
D-6900 Heidelberg

isoquinoline, no. 802406,
dimethylsulfoxide (DMSO),
no. 2931,
E. Merck,
D-6100 Darmstadt 1

helium 5.0,
nitrogen,
air (synthetic),
hydrogen,
Linde AG,
D-5000 Köln

calibration solutions:
0.4, 3, and 8 g/l nicotine in DMSO containing 0.1 % (v/v) triethylamine

internal standard solution:
0.5 g/l isoquinoline in n-butylacetate containing 0.1 % (v/v) triethylamine

Procedure: addition of 9 ml internal standard solution to 1 ml condensate suspension in DMSO in a centrifuge tube, shaking for 10 s, 5 min centrifuged at 7.8E3 m/s2 (= 820 x g), 1 µl used for gas chromatography

Gas chromatography

Column:	15 m x 0.25 mm inner diameter, fused silica
Stationary phase:	SE-54, chemically bonded, film thickness: 0.25 µm
Carrier gas and flow rate:	helium, 1.5 ml/min at 150 °C
Detector:	FID, 250 °C
Injector:	split injector: 220 °C, split ratio 1 : 50

Make up gas:	nitrogen, 30 ml/min
Septum purge:	3 ml/min
Injection volume:	1 µl
Oven temperature:	150 °C, isothermal
Retention times:	isoquinoline: 1 min, nicotine: 1.3 min
Computation:	nicotine standard solutions treated in the same way as a sample, construction of a calibration curve using the data system with internal standard method
Reproducibility (RSD)	1.6 % (sidestream condensate suspension of cigarette 2R1, nicotine concentration: 6.2 g/l, N = 5)
Scientific version: Text version:	SOP AC 120/1 17 Aug.90

5.2.3.3 Determination of humectants in filler and whole smoke condensate

Principle:	gas chromatographic determination of humectants in methanolic extract of filler and in DMSO suspension of whole smoke condensate (a) data acquisition and evaluation using a laboratory data system
Time:	within 1 week after extraction of filler or preparation of whole smoke condensate suspension

(a) Propylene glycol cannot be determined in WSC-I/DMSO suspension due to interference with DMSO.

Sample material and quantity:	filler 5.0 g, WSC-I/DMSO suspension, 1.0 ml
Results expressed in:	mg/cigarette
Equipment:	capillary gas chromatograph: Carlo Erba, 5300 Mega Series, detector: FID, autosampler AS 550, Carlo Erba Instruments, D-6238 Hofheim capillary column: Carbowax 20M, no. 19091-61125, Hewlett-Packard GmbH, D-7030 Böblingen laboratory data system: hardware: Microvax II, VT 340, LA210, Digital Equipment GmbH, D-8000 München software: Multichrom, VG Instruments GmbH, D-6200 Wiesbaden centrifuge: model J6-B, rotor: JS-4.2, Beckmann Instruments GmbH, D-8000 München 40 mechanical shaker, centrifuge tube 100 x 20 mm, Faust GmbH, D-5000 Köln 90
Chemicals:	DMSO, no. 2950, methanol, no. 6009, isopropanol, no. 9634, glycerol, no. 4093, E. Merck, D-6100 Darmstadt 1

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triethylene glycol, no. T5,945-5, propylene glycol, no. 13,436-8, Aldrich-Chemie GmbH und Co. KG, D-7924 Steinheim

calibration solution: 1.90 g/l 1,2-propandiol, 1.96 g/l triethylene glycol, and 1.97 g/l glycerol in methanol, diluted with isopropanol to appropriate concentrations before use

hydrogen, nitrogen, air (synthetic), Linde AG, D-5000 Köln 50

Procedure: 10 ml methanol added to 0.5 g filler in a centrifuge tube, mechanically shaken for 2 h, after standing overnight again shaken for 1 h, centrifuged, 1 µl methanolic extract injected into the gas chromatograph, 1 µl DMSO suspension of WSC-I injected into the gas chromatograph, samples diluted with isopropanol if necessary

Gas chromatography

Column: 25 m x 0.32 mm inner diameter, fused silica

Stationary phase: Carbowax 20M

Carrier gas: hydrogen, 50 kPa, corresponding 4.1 ml/min at 80 °C

Oven temperature: initial temperature 80 °C kept for 1.0 min, increase 30 °C/min to 220 °C, final temperature kept for 5 min

Detector temperature:	300 °C
Injection port temperature:	250 °C
Injection:	1 µl, split ratio 1 : 15
Retention times:	approx. 3.1 min for 1,2-propandiol, 5.9 min for glycerol, and 6.0 min for triethylene glycol
Computation:	construction of a calibration curve from peak area versus humectant concentration and evaluation by the external standard method using the laboratory data system
Scientific version:	SOP AC 86/2
Text version:	24 Sep.90

5.2.3.4 Bacteriological examination of WSC-I/DMSO suspension

Principle:	determination of bacterial contamination of test substance assayed for mutagenicity in the plate incorporation assay
	detection limited to aerobic bacteria growing on minimal-glucose agar plates
Time:	on the day of mutagenicity assay
Sample material and quantity:	WSC-I/DMSO suspension, highest dose/plate, 1 WSC-I batch of each test cigarette, condensate type, and substudy
Results expressed in:	CFU/plate

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Equipment:	incubator: no. 3916, Forma Scientific, via Labotect, D-3400 Göttingen petri dishes: no. 1029, 100 mm x 15 mm, polystyrene, sterilized, Falcon, via Becton Dickinson GmbH, D-6900 Heidelberg 1 colony counter: Colony Star 2, Funke-Gerber, D-1000 Berlin
Chemicals:	top agar and minimal-glucose agar, composition: see Chapter 5.6.1
Procedure:	top agar and test substance mixed by rotation and poured on minimal-glucose agar plates, 2 plates/ sample incubation of plates at 37 °C, manual counting of colonies after 2 d of incubation
Scientific version: Text version:	SOP MB 44/1 27 Oct.87

5.3 Dosing of Test Substances

Principle:	dilution of test substance stock suspension with DMSO to the final concentration used in the study
Time:	on the day of mutagenicity assay
Sample material and quantity:	4 WSC-I suspension batches/condensate type

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Equipment:	whirlmix: no. 9.730130, Heidolph Elektro GmbH und Co. KG, D-8420 Kelheim
	brown glass vials, 8 ml, no. 224984, screw caps, no. 240409, Wheaton Scientific, via Zinsser, D-6000 Frankfurt/Main
Chemicals:	DMSO, no. 2950, E. Merck, D-6100 Darmstadt 1

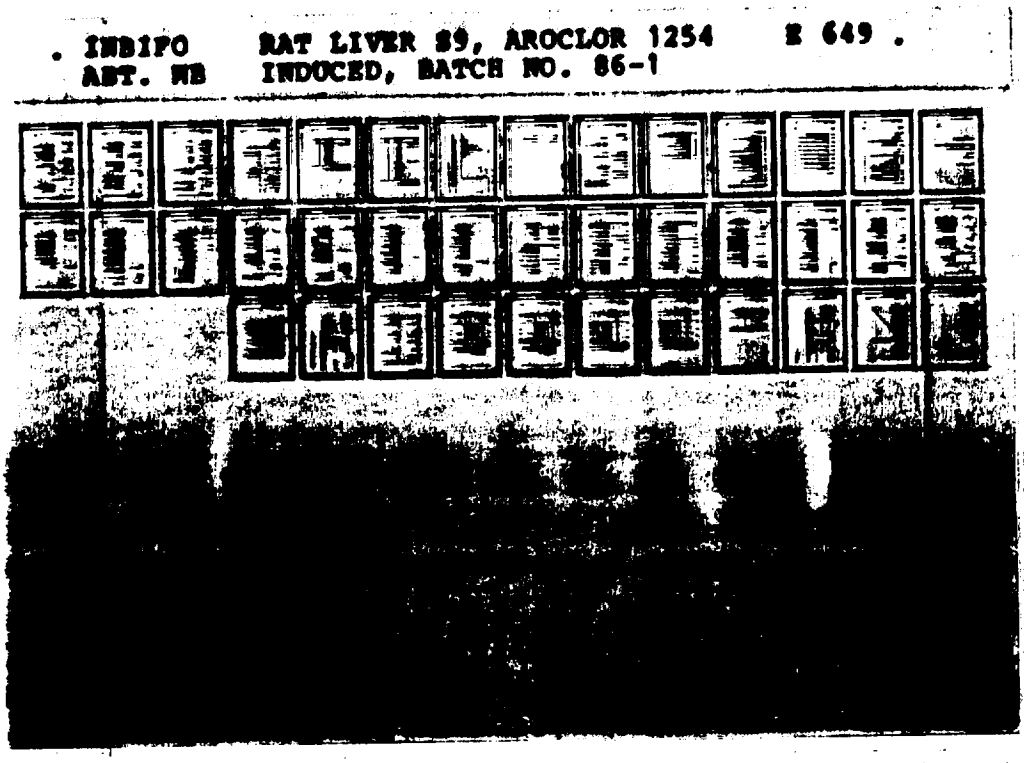
Procedure

Preparation of application suspension:	shaking of WSC-I/DMSO stock suspension on a whirlmix
	2 application suspensions/condensate batch, 2.5 g dry cond./l
Storage:	in dark airtight vials at RT
Dosing:	see TABLE 2
Scientific version:	SOP MB 43/2
Text version:	9 Jan.89

5.4 Metabolic Promutagen Activation System

Principle:	metabolic promutagen activation system consisting of a postmitochondrial (S9) fraction from the livers of rats treated with Aroclor 1254 and a NADPH-generating system
Time:	mixing of S9 protein and NADPH-generating system on the day of mutagenicity assay

Sample material and quantity:	S9 fraction, batch no. 86-1, approx. 2 mg protein/plate
Analyses:	determination of protein concentration, AHM activity, and bacterial contaminants interfering with the mutagenicity assay
Standard operating procedure:	see attached microfiche no. E649



Scientific version:	SOP AT 21/6, AT 26/2, AT 73/2, AT 76/2, AT 102/3, BC 1/13, BC 128/2, MB 1/2, MB 46/4, MB 47/5, MB 48/1, MB 49/1
Text version:	22 Aug.90

B.5 Tester Strain Bacteria

B.5.1 Species and source

Species:	Salmonella typhimurium LT-2 mutant strains TA98 and TA100
Genotypes	
hisD3052 or hisG46:	mutations in histidine operons, resulting in histidine requirement (TA98: hisD3052, TA100: hisG46)
rfa:	deep rough, mutation in the lipopolysaccharide barrier making the bacteria cell more permeable and completely nonpathogenic
delta uvrB:	deletion of excision repair system resulting in sensitivity to ultraviolet light
pKM101:	resistance transfer system, so-called R factor plasmid
Sensitivity	
TA98:	to mutagens causing frameshift mutation
TA100:	to mutagens causing base-pair substitution
Source:	kindly provided by Prof. Dr. Bruce N. Ames, University of California, Berkely CA, U.S.A
Receipt at INBIFO:	13 Jul.79
Text version:	16 Oct.87

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5.5.2 Cultivation

Principle:	cultivation of tester strain bacteria in nutrient broth to an early stationary growth phase
Time:	approx. 12 h before use in the mutagenicity assay
Sample material and quantity:	Salmonella typhimurium strains from stock culture stored at -196 °C
Results:	cultures of tester strain bacteria
Equipment:	incubator shaker: model G 24, New Brunswick Scientific, via Biotronik, D-4000 Düsseldorf culture flask: Erlenmeyer flask, 100 ml, with long neck and 4 baffles, used with stainless steel caps, Schott, D-6500 Mainz vial for cold storage: polypropylene, 1 ml, with screw caps, no. 985730, Wheaton Scientific, via Zinsser, D-6000 Frankfurt/Main photometer: DB-GT, Beckman Instruments GmbH, D-8000 München 40 disposable plastic cuvets, no. 127-1010-400, Elkay Products, via Nunc GmbH, D-6200 Wiesbaden 12

Chemicals:	DMSO, for spectroscopy, no. 2950, E. Merck, D-6100 Darmstadt 1
	Difco-nutrient broth, no. 0003, Difco Laboratories, via Biotest AG, D-6072 Dreieich
	PBS without Mg2+ and Ca2+, no. L1825, Biochrome KG, D-1000 Berlin 46

Procedure

Inoculation:	addition of 10 µl of the thawed and 10-fold diluted stock culture to 30 ml nutrient broth in the culture flask
Cultivation:	cultures incubated in a shaking incubator at 37 °C at 200 rpm
	cultures grown for approx. 12 h to obtain an early stationary growth phase
	growth phase determined photometrically at 565 nm as cell suspension density from 5-fold diluted culture suspension in PBS, absorbance calculation for the undiluted culture
Centrifugation:	no centrifugation and no washing of bacterial cells
Storage of stock culture:	at -196 °C in liquid nitrogen in 0.1 ml aliquots of tester strain suspension culture with 87.5 ml DMSO/l

Scientific version:	SOP MB 50/3, MB 51/4
Text version:	10 Mar.89

5.3 Determination of number of viable bacteria

Principle:	spreading of bacteria with top agar plating technique and counting of colony-forming units
Time:	at the start and at the end of the mutagenicity assay
Sample material and quantity:	bacteria suspension culture, approx. 0.1 ml of each strain
Results expressed in:	CFU/plate
Equipment:	see Chapter 5.6.1
Chemicals:	nutrient agar, standard 1, no. 7881, E. Merck, D-6100 Darmstadt 1
	L-histidine hydrochloride-1-hydrate, no. H 8125, biotin, no. B 4501, Sigma Chemie GmbH, D-8024 Deisenhofen
	PBS without Mg^{2+} and Ca^{2+}, no. L1825, Biochrome KG, D-1000 Berlin 46
	histidine/biotin solution no. 2: 20.96 g histidine hydrochloride-1-hydrate and 244 mg biotin dissolved in 1 l distilled water, sterilized by filtration
	minimal-glucose agar and top agar, composition: see Chapter 5.6.1

rocedure:	aliquots of bacteria suspension culture diluted 1E6-fold in PBS, 0.1 ml of this dilution mixed with 2.0 ml top agar and 0.1 ml histidine/biotin solution (omitted on nutrient agar) and plated on minimal-glucose agar or nutrient agar plates incubation: approx. 24 h (nutrient agar) or 44 to 48 h (minimal-glucose agar) at 37 °C counting of CFU: see Chapter 5.6.1
cientific version: ext version:	SOP MB 53/4 10 Mar.89

5.5.4 Analyses of tester strain properties

Principle:	tester strain checked for: (1) auxotrophy in the form of histidine requirement (2) absence or presence of lipopolysaccharide barrier in the form of sensitivity or resistance to crystal violet (3) absence or presence of excision repair system in the form of sensitivity or resistance to ultraviolet light (4) absence or presence of R factor in the form of sensitivity or resistance to ampicillin
Time:	prior to and at the end of the study
Sample material and quantity:	bacteria suspension culture, approx. 0.5 ml

Equipment: ultraviolet light source:
Astralux F, no. 15136, 890 W,
Astralux-Werke,
A-1000 Wien

filter paper disks: no. 95354,
ampicillin sensitivity disk,
10 µg/disk, no. 93332,
diameter: 6 mm,
Becton Dickinson GmbH,
D-6900 Heidelberg 1

incubator: no. 3916,
Forma Scientific,
via Labotect,
D-3400 Göttingen

Chemicals: L-histidine hydrochloride-1-hydrate,
no. H 8125,
biotin, no. B 4051,
Sigma Chemie GmbH,
D-8024 Deisenhofen

crystal violet, no. 1407,
nutrient agar, standard 1,
no. 7881,
E. Merck,
D-6100 Darmstadt 1

histidine/biotin solution no. 2:
20.96 g histidine hydrochloride-1-hydrate and 244 mg biotin dissolved in 1 l distilled water, sterilized by filtration

crystal violet solution:
1 g dissolved in 1 l H2O

minimal-glucose agar composition:
see Chapter 5.6.1

Procedure

Reference: basically according to Maron and Ames (1983)

Histidine requirement:	tester strain bacteria streaked on minimal-glucose agar plates without and with 0.1 ml of histidine/biotin solution no. 2, incubation at 37 °C for 18 to 24 h, plates checked for growth
Crystal violet sensitivity:	10 µl crystal violet solution applied to filter paper disk, placed onto complete nutrient agar plate with tester strain bacteria plated, incubation at 37 °C for 12 to 16 h, plates checked for inhibition or growth zone around the disk
Sensitivity to ultraviolet light:	tester strain bacteria to be tested streaked across nutrient agar plates and half of the streak irradiated for 30 s with ultraviolet light at a distance of 33 cm, incubation at 37 °C for 18 to 24 h, plates checked for growth inhibition
Ampicillin sensitivity:	ampicillin disk applied onto nutrient agar plates with tester strain bacteria plated, incubation at 37 °C for 18 to 24 h, plates checked for growth or inhibition zone around ampicillin disk
Scientific version:	SOP MB 54/5
Text version:	1 Sep.88

5.6 Mutagenicity Assay

5.6.1 Plate incorporation assay

Principle:	mixture of test substance and tester strain bacteria with or without the metabolic activation system spread on minimal-glucose agar plates containing minimal amounts of histidine and biotin using the top agar plating technique after incubation at 37 °C counting of revertants (see FIGURE 4)
Time of top agar plating:	15 and 16 Aug.89
Sample material and quantity:	(1) research substances: MWSC-I and SWSC-I of research cigarettes, see TABLES 1 and 2 (2) internal controls: MWSC-I and SWSC-I of the standard reference cigarette 2R1, see TABLE 3 (3) positive controls: diagnostic mutagens, see TABLE 4
Results expressed in:	revertants/plate and increase in the number of revertants/mg dry condensate
Equipment:	medium autoclave: cultmatic 800 with Pretagar controller, Best, via E. Schütt jr., D-3400 Göttingen petri dish filler: automatic dose dishes (Tecnopront 100, Tecnomat 125), printer (Tecnoprint 300), and stacking unit (Stacomat 501), Tecnomara Deutschland GmbH, D-6301 Fernwald

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test tubes:
sodium lime silicate glass,
16 mm x 100 mm,
no. 114115,
Rudolf Brand GmbH und Co.,
D-6980 Wertheim

petri dishes:
no. 1029, 100 mm x 15 mm,
polystyrene, sterile,
Falcon,
via Becton Dickinson GmbH,
D-6900 Heidelberg 1

disposable membrane filter unit:
Millex, 0.45 µm pore size,
no. SLHA 025BS (for S9 mix),
filter unit: sterifil,
no. XX1104710,
prefilter, no. AP 2504200,
membrane filter, no. HAWG 04700,
0.45 µm pore size (for glucose solution),
Millipore GmbH,
D-6078 Neu-Isenburg

disposable membrane filter unit:

(1) 0.2 µm pore size, no. FP 030/3
(for NADP and G6P),
Schleicher und Schüll GmbH,
D-3354 Dassel

(2) 0.2 µm pore size, no. 120-0020
and no. 450-0020 (for
histidine-biotine solution),
Nalge Company,
via Faust GmbH,
D-5000 Köln 90

incubator: no. 3916,
Forma Scientific,
via Labotect,
D-3400 Göttingen

whirlmix: no. 9.730130,
Heidolph Elektro GmbH und Co. KG,
D-8420 Kelheim

thermostat:
aluminum bloc thermostat,
no. 2092,
Gebr. Liebisch,
D-4800 Bielefeld

hand-hold terminal: micronic,
no. 445 AA,
Facit AB,
with hand-hold laser scanner,
LS 8110 II,
Symbol Technologies, Inc.,
via Parcon GmbH,
D-4030 Ratingen

automatic colony counter connected via micronic to IBM personal computer XT:
model no. 880,
Artek System Corporation,
via Fisher Scientific,
D-8000 München

manual colony counter:
Colony Star 2,
Funke-Gerber,
D-1000 Berlin

automatic pipettes:

(1) refilling syringes:
Cornwall syringe,
max. volume: 2 ml (for top agar),
Becton Dickinson GmbH,
via E. Schütt,
D-3400 Göttingen

(2) bottle-top dispenser:
"dispensette", max. volume:
2 ml (for S9 mix),
Brand GmbH und Co.,
D-6980 Wertheim

"distrivar", max. volume: 5 ml
(for bacteria suspension),
Gilson,
via Abimed,
D-4000 Düsseldorf

(3) adjustable pipettes:
P 20, P 100, and P 1000,
max. volumes: 0.02, 0.1,
and 1 ml (for test substance),
Gilson,
via Abimed,
D-4000 Düsseldorf

"finnpipette" digital,
no. F 4027-010, max. volume:
0.04 ml (for test substance),
"Justor 1100DG" digital,
no. F11DG-50,
max. volume: 5 ml (for
solvent),
LKB Instrument GmbH,
D-8032 Gräfelfing

Chemicals:

glucose-6-phosphate-disodium,
no. 127647,
NADP-disodium, no. 128058,
Boehringer Mannheim GmbH,
D-6800 Mannheim 31

agar, no. 1614,
citric acid-1-hydrate, no. 244,
DMSO, no. 2950,
D(+)-glucose-1-hydrate, no. 8342,
magnesium chloride-6-hydrate,
no. 5833,
magnesium sulfate-7-hydrate,
no. 5886,
sodium ammonia hydrogen phosphate-
4-hydrate, no. 6682,
sodium chloride, no. 6400,
sodium dihydrogen
phosphate-1-hydrate, no. 6346,
disodium hydrogen
phosphate-2-hydrate, no. 6580,
potassium chloride, no. 4936,
dipotassium hydrogen
phosphate-3-hydrate, no. 5099,
E. Merck,
D-6100 Darmstadt 1

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2-aminoanthracene, no. A 1381,
2-aminofluorene, no. A 9031,
daunomycin, no. D 4885,
methyl methanesulfonate,
no. M 4016,
D(+)-biotin, no. B 4501,
L-histidine-hydrochloride-
1-hydrate, no. H 8125,
Sigma Chemie GmbH,
D-8024 Deisenhofen

nitrogen, ≥99.999 % (v/v),
Linde AG,
via Elbert,
D-5000 Köln

Minimal-glucose agar:

composition (g/l):

glucose-1-hydrate	20.0
magnesium sulfate-7-hydrate	0.2
dipotassium hydrogen phosphate-3-hydrate	13.1
citric acid-1-hydrate	2.0
sodium ammonia hydrogen phosphate-4-hydrate	3.5
agar	15.0

glucose solution prepared separately 10-fold concentrated and sterilized by filtration including prefilter, salts 10-fold concentrated and agar solutions sterilized separately at 121 °C (1.0E5 Pa) for 15 min

automatically filling into petri dishes, approx. 30 ml molten agar/plate, excess water on the solid agar plates removed by exposure of the covered plates at 37 °C for approx. 3 d, subsequently stored at RT

Top agar:

composition (g/l):

L-histidine-hydrochloride-1-hydrate	0.0095
biotin	0.011
sodium chloride	4.5
agar	5.5

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histidine-biotin solution prepared separately as 10-fold concentrated solution (no. 1: 0.5 mmol histidine-hydrochloride-1-hydrate and 0.5 mmol biotin/l), filter-sterilized, and stored at 4 °C

agar-sodium chloride solution sterilized at 121 °C (1.0E5 Pa) for 15 min and stored at RT

prior to use remelting of the agar-sodium chloride solution by boiling in a water bath or autoclave for approx. 20 min, addition of histidine-biotin solution (no. 1) after cooling down to approx. 45 °C

S9 mix (a):

composition (g/l and mmol/l):

sodium phosphate buffer, pH 7.4	-	100.0
magnesium-chloride-6-hydrate	3.25	16.0
potassium chloride	4.92	66.0
glucose-6-phosphate-disodium	1.68	5.0
NADP-disodium	3.22	4.0
S9 protein (prior to filtration)	10.0	-

S9 mix filter-sterilized prior to use and stored at 0 °C under nitrogen atmosphere during the mutagenicity assay

dilution of S9 mix with sodium phosphate buffer (0.1 mol/l) to adjust other S9 protein concentrations with a fixed S9 protein/cofactor ratio (according to Zeiger et al., 1979)

(a) protein concentration and AHM activity determined from each S9 mix

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	sodium phosphate buffer, pH 7.4, 0.1 mol/l (prepared as a 2-fold concentrated solution, 5.24 g sodium dihydrogen phosphate/l and 28.8 g disodium hydrogen phosphate/l), sterilized at 121 °C (1.0E5 Pa) for 15 min and stored at 4 °C
	magnesium-potassium chloride prepared as 25-fold concentrated solution, sterilized at 121 °C (1.0E5 Pa) for 15 min and stored at 4 °C
	glucose-6-phosphate prepared as 200-fold and NADP as 25-fold concentrated solutions, filter-sterilized, and stored at -75 °C
Tester strain bacteria:	Salmonella typhimurium strains TA98 and TA100, approx. 12-h cultures grown in Difco nutrient broth
Procedure	
Reference:	basically according to Maron and Ames (1983)
Dosing of test substance:	see TABLE 2
Plating mixture preparation:	components added in the following order: (1) 2.0 ml top agar, 45 °C (2) research substance, stored at RT (3) 0.1 ml tester strain suspension culture, containing approx. 1E8 CFU, stored at RT (a) (4) 0.5 ml S9 mix or buffer solution stored at 0 °C under nitrogen atmosphere

(a) Temperature may rise to approx. 30 °C during mutagenicity assay.

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Top agar plating:	components mixed by rotating the test tube gently on a whirlmix, then poured on minimal-glucose agar plates and spread evenly on the agar surface by wobbling mixing, pouring, and spreading of the top agar occurred within 20 s, plates allowed to harden for 3 to 6 min and then transferred to the dark incubator
Incubation:	44 to 48 h at 37 °C in the dark
Labeling of the petri dishes:	individual plate no. including bar code (type 2 of 5 interleaved), project no., name of test substance, batch no., type of test substance, tester strain, absence/presence of metabolic activation system, test substance doses, date of top agar plating
Counting of revertants:	manual and/or automatic counting immediately or after storage at 4 °C for not longer than 48 h, plates brought to RT automatic counting: standardized by 5100 mm2 plate aperture area without discrimination of colony size, each plate counted 3 times, rotation of the plate 120° between each count, data recorded on floppy disk, highest count used for the computer calculation of revertants
Scientific version:	SOP MB 55/4
Text version:	30 May 90

5.6.2 Statistical evaluation

Primary data (revertants/plate): calculation of mean, SE, and RSD from all plates of each dose for each research cigarette, also calculated separately for each substudy and WSC-I batch, data not corrected for automatic counting

data stored and calculated in data base management system ORACLE on a VAX computer

specific mutagenicity (a): increase in the number of revertants per mg dry condensate (rev./mg dry cond.)

equivalent to regression coefficient "a" of the linear dose-response curve
$y = ax + b$

mutagenicity on a "new tar" basis:

$$x = \text{spec. mutagen.} \times \frac{\text{dry cond. yield}}{\text{dry cond. yield - nicotine yield - humectant yield}}$$

Relative difference: absolute difference between 2 values (A and B) divided by the mean of them

$$\frac{|A - B|}{(A + B)/2}$$

(a) The specific mutagenicity derived from the regression curve might be slightly different from the mean of 4 condensate batches calculated separately.

Reproducibility of the specific mutagenicity of substudy 1 in substudy 2:	statistical significance of the difference between the specific mutagenicities obtained in 2 independent substudies fixed at a level of significance of alpha = 0.017 with a relative difference between the specific mutagenicities of 25 % (a) reproducibility presumed if level of significance not reached
Statistical significance of the difference between the specific mutagenicities of MWSC of 2 research cigarettes:	statistical significance reached at the level of significance alpha = 0.05 with a relative difference between the specific mutagenicities of 16 % (a)
Scientific version:	SOP MB 60/2
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(a) In the basic biometric INBIFO study P 0268/2029 with strain TA98, the MWSC-I of 1 test cigarette was assayed according to the INBIFO standard operating procedure (same procedure as in the present study: 2 independent substudies, 4 doses, and 64 plates/test cigarette). A basic biometric study with strain TA100 not having been performed, the limit of the relative difference for 2 test cigarettes or 2 substudies is set at 0.16 or 0.25 respectively as in the biometric study with strain TA98.

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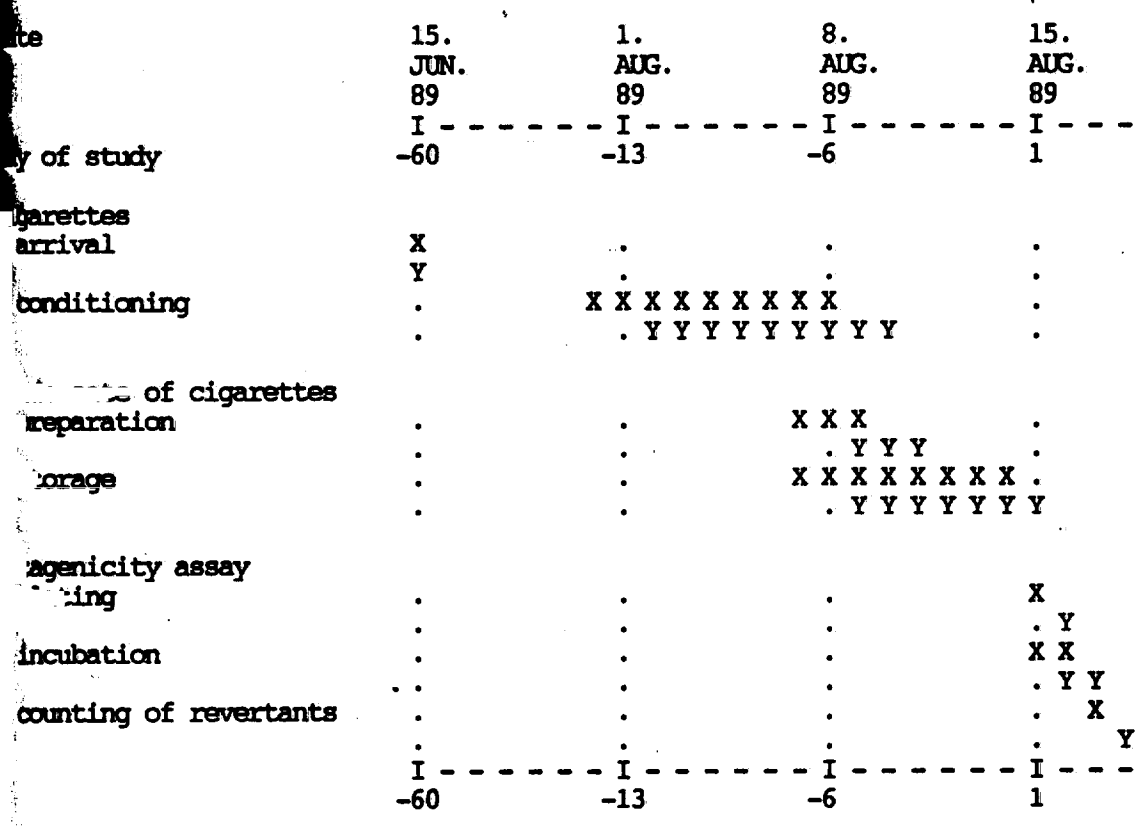

TRE 1 CHRONOLOGY

marks: X: substudy 1
Y: substudy 2

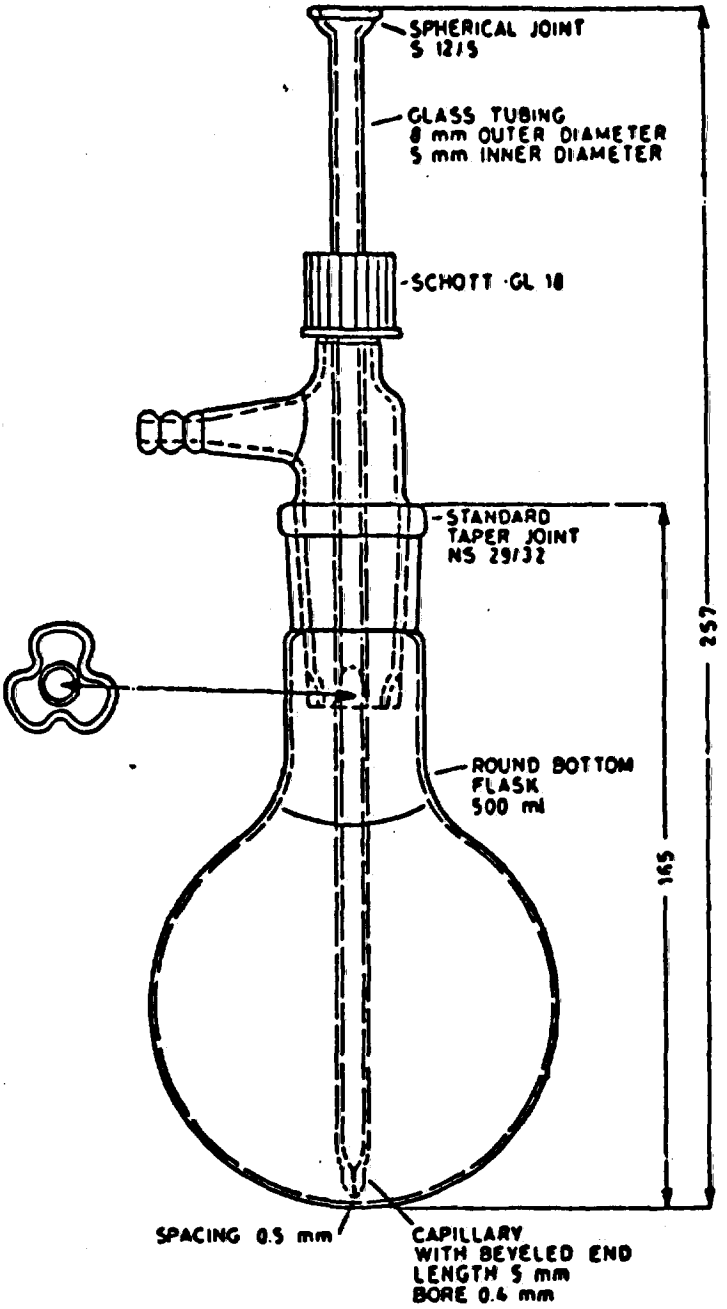

FIGURE 2 GLASS IMPACTION TRAP FOR MWSC-I COLLECTION

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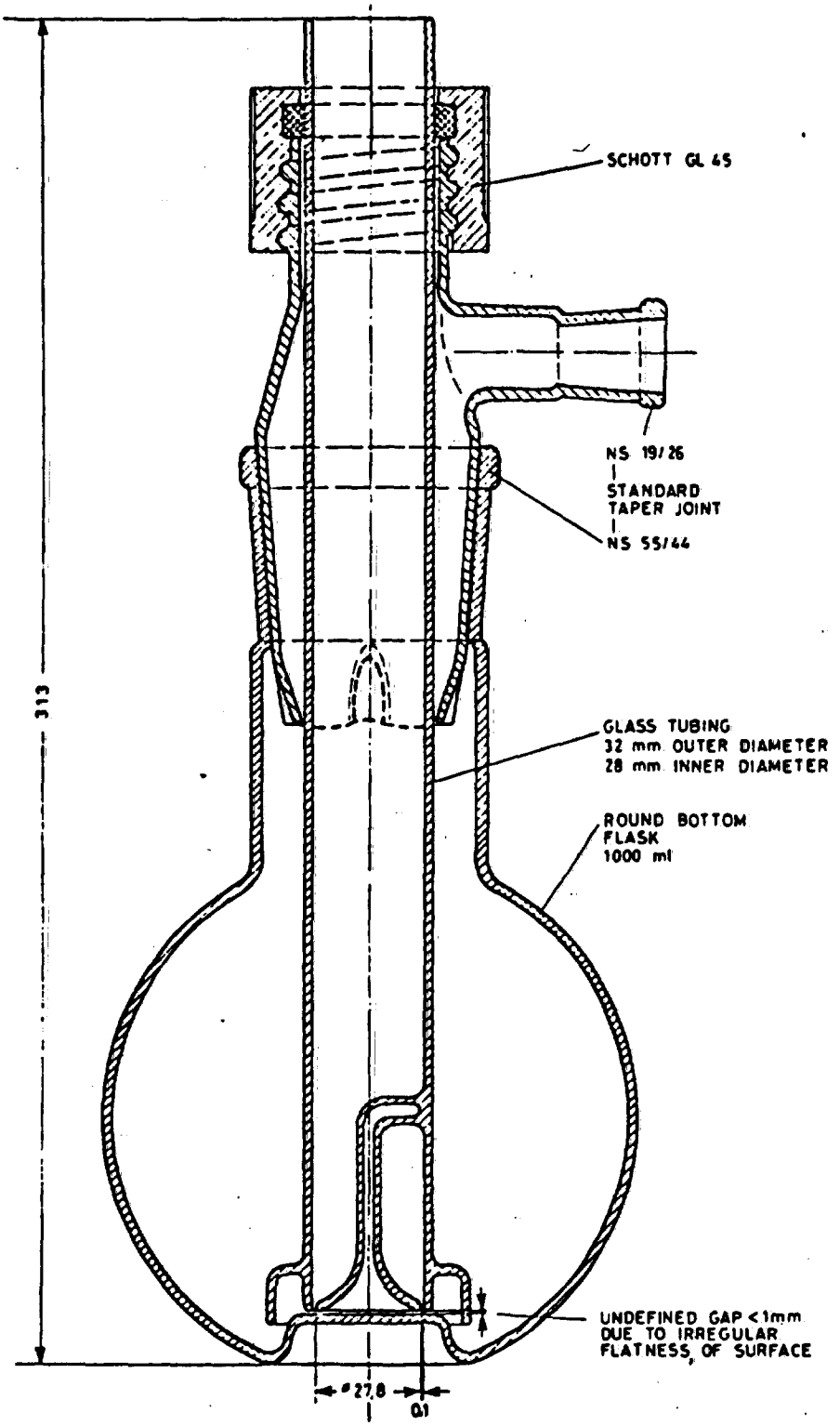

FIGURE 3 GLASS IMPACTION TRAP FOR SWSC-I COLLECTION

RESEARCH SUBSTANCE	METABOLIC ACTIVATION	APPLICATION SUSPENSION CONCENTRATION (g dry cond./l)	APPLICATION SUSPENSION VOLUME PLATED (µl/plate)	DOSE (mg dry cond./plate)
WSC-I, BWSC-I	yes	2.5	0	0 (a)
			20	0.050
			40	0.100
			60	0.150

TABLE 2 DOSING OF RESEARCH SUBSTANCES

Remarks: Of each WSC-I batch and for each activation system, 2 application suspensions were prepared using DMSO as solvent.

(a) solvent control: 60 µl DMSO/plate

YPE OF ONDEN-ATE	TESTER STRAIN	SPECIFIC MUTAGENICITY (rev./mg dry cond.) N	M ± SD	MIN.	MAX.
WSC-I	TA98	61	1501 ± 193	907	1854
	TA100	49	885 ± 165	559	1400
WSC-I	TA98	27	1540 ± 170	1288	1896
	TA100	27	1247 ± 256	899	2278

ABLE 3 HISTORICAL DATA OF THE SPECIFIC MUTAGENICITY OF MWSC-I AND SWSC-I OF THE STANDARD REFERENCE CIGARETTE 2R1, TIME PERIOD: JUL.80 TO AUG.89

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TESTER STRAIN	PARAMETER	PRESENCE OF S9	DOSE PER PLATE	HISTORICAL DATA (rev./plate) N	M ± SD	MIN.	MAX.	PUBLISHED DATA (rev./plate)
TA98	spont. rev.	no	-	17	24.2 ± 3.2	19.0	30.8	30 to 50 (1)
		yes	-	17	42.6 ± 6.8	33.0	55.8	-
	daunomycin	no	6 µg	16	1198 ± 207	792	1595	approx. 1020 (2)
	2-AA	yes (a)	2 µg	16	1462 ± 171	1091	1910	approx. 1000 (3)
	2-AF	" (a)	2 µg	16	289.6 ± 71.6	132	415	approx. 140 (1)
TA100	spont. rev.	no	-	17	138.9 ± 19.2	105.5	173.5	120 to 200 (1)
		yes	-	17	134.1 ± 21.9	102.3	190.0	-
	MMS	no	0.5 µl	17	600.3 ± 130.6	474	863	approx. 1360 (1)
	2-AA	yes (a)	2 µg	17	1847 ± 156	1441	2020	-
	2-AF	" (a)	2 µg	17	173.4 ± 34.8	142	295	approx. 280 (1)

TABLE 4 HISTORICAL DATA OF SPONTANEOUS AND INDUCED REVERTANTS BY DIAGNOSTIC MUTAGENS, TIME PERIOD: JUN.85 TO DEC.87

Remarks: (1) Maron and Ames (1983)
(2) Babudri et al. (1984)
(3) Zeiger et al. (1979)
data corrected for spontaneous revertants

(a) S9 amount not optimized

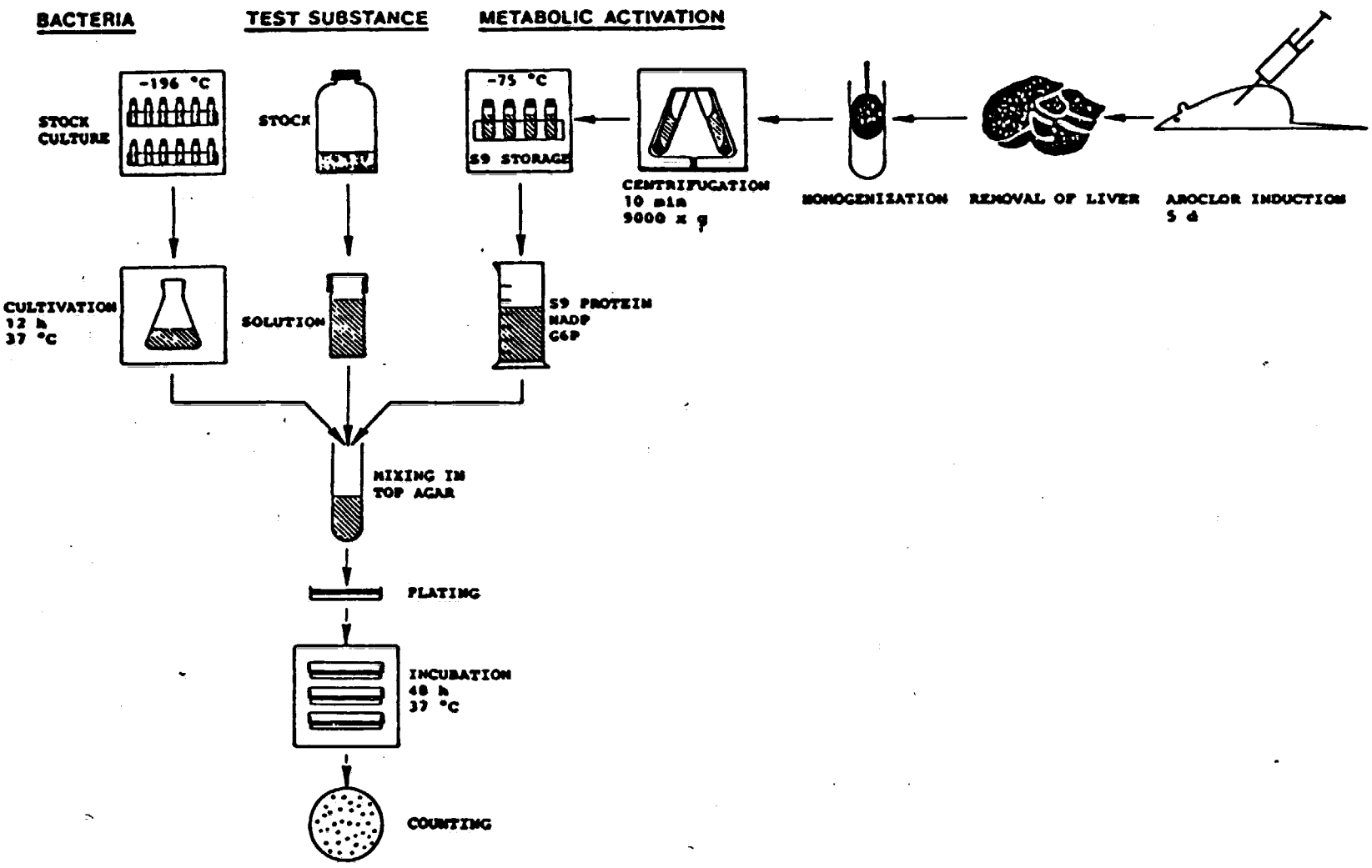

FIGURE 4 FLOW CHART OF THE PLATE INCORPORATION MUTAGENICITY

STORAGE OF MATERIALS AND RECORDS

esearch substance

Test cigarettes:	no storage after completion of the study
Condensates:	approx. 5 ml WSC-I stock suspension of each condensate preparation transferred on day of cigarette condensate preparation into brown glass vial with screw cap and stored at -75 degrees centigrade for at least 1 year the remaining volume of each condensate preparation stored at 4 degrees centigrade for approx. 1 month
Protocol, records, and valuation sheets:	stored in our archives for at least 5 years after delivery of the final report to the client. They can be claimed by the client.
Scientific version:	SOP MB 61/1, QA 10/4
Text version:	30 May 90

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RESULTS AND COMMENT

.1 Text

.1.1 Test substance

n the filler the propylene glycol concentration was the same in ll research cigarettes (see TABLE 5). The test cigarette REUSE-53 was found to contain triethylene glycol, and AREUSE-55 as the cigarette having the highest glycerol concentration of the esearch cigarettes.

In the condensates propylene glycol could not be determined because it could not be separated from the solvent DMSO. The glycerol yield of the MWSC-I was approx. 1.3 milligrams per cigarette for CALYPSO-1, AREUSE-46, and -53 and 3.6 milligrams per cigarette for AREUSE-55 (see TABLE 6). For the SWSC-I, it was approx. 1.8 and 6.3 milligrams per cigarette (see TABLE 7). The triethylene glycol yield of the MWSC-I was <0.1 milligrams per cigarette for CALYPSO-1, AREUSE-46, and -55 and 1.0 milligram per cigarette for AREUSE-53 (see TABLE 6). For the SWSC-I, it was ≤0.1 and 1.2 (see TABLE 7). These humectant yields correlated with the concentrations determined in the filler.

The dry condensate yield of the MWSC-I was approx. 17 milligrams per cigarette for all research cigarettes (see TABLE 8 and FIGURE 5). The dry condensate yield of the SWSC-I was higher ranging from 22 milligrams per cigarette for CALYPSO-1 to 28 milligrams per cigarette for AREUSE-55 (see TABLE 9 and FIGURE 5).

The application suspensions of MWSC-I and SWSC-I of the research cigarettes were found to be free of bacterial contaminants which could interfere with the mutagenicity assay (see TABLE 10).

11.2 Properties of the tester strains and the S9 fraction

The strains were found to respond in a manner characteristic of their phenotypes as required in the basic "method paper" by Maron and Ames (1983) (see TABLES 11 and 14).

The number of spontaneous revertants of strain TA98 was found to be slightly lower than that obtained in previous studies and that reported by Maron and Ames (1983) (see TABLES 4, 13, and 16). This is considered to be caused by the use of an agar from another supplier. However, the response of both tester strains to diagnostic mutagens and to MWSC-I and SWSC-I of 2R1 as internal controls was in accordance with results obtained in previous studies (see below).

The mutagenic activity of the strain-specific positive control substances daunomycin (TA98) and MMS (TA100) was found to be in accordance with the results obtained in previous studies and those published by Babudri et al. (1984) and Maron and Ames (1983) (see TABLES 4, 13, and 16). The mutagenic response of both tester strains to the promutagens 2-AA and 2-AF indicated that the metabolic activity of the S9 was sufficient and was in accordance with the data published by Zeiger et al. (1979) and Maron and Ames (1983).

The amount of S9 protein per plate was 1.6 milligrams (see TABLE 17). The AHM activity of the S9 mix was found to be approx. 45 percent higher than the activity previously determined in the original S9 fraction (see Chapter 5.4, microfiche no. E649). This increase might be due to the protein determination which was performed according to Lowry which always yields a lower protein concentration than the previously used Biuret method.

7.1.3 Mutagenicity of whole smoke condensate

7.1.3.1 Dose response and reproducibility

An approx. linear increase in the number of revertants with increasing doses of WSC-I was obtained with respect to frameshift mutation in TA98 and base-pair substitution in TA100 (see TABLES 18 to 37 and FIGURES 6 to 13).

The specific mutagenicity of the MWSC-I and SWSC-I of all research cigarettes determined in substudy 1 was reproduced in substudy 2 (relative difference ≤0.25, range 0.01 to 0.18) (see "Remarks" in TABLES 8 to 37).

The specific mutagenicity of the MWSC-I and SWSC-I of the standard reference cigarette 2R1 was found to be in the range expected for frameshift mutation induction in strain TA98 and base-pair substitution induction in strain TA100 when compared with all previous INBIFO data (see TABLES 3, 22, 27, 32, and 37).

7.1.3.2 Specific mutagenicity of MWSC-I

With respect to frameshift mutation in strain TA98, the mean activity of MWSC-I was found to be 2180 revertants per milligram dry condensate for the cigarette CALYPSO-1, 2401 for AREUSE-46, 2317 for -53, and 2119 for -55 (see TABLE 38 and FIGURE 14).

With respect to base-pair substitution in strain TA100, the mean activity of MWSC-I was found to be 984 revertants per milligram dry condensate for the cigarette CALYPSO-1, 1009 for AREUSE-46, 955 for -53, and 867 for -55 (see TABLE 38 and FIGURE 14).

e specific mutagenicity of the MWSC-I of the test cigarette REUSE-55 was numerically lower than that of the reference igarette AREUSE-46 with respect to both types of mutation (see ABLE 39). MWSC-I of CALYPSO-1 was less mutagenic than that of REUSE-46 with respect to frameshift mutation and more mutagenic han that of AREUSE-55 with respect to base-pair substitution. owever, none of these differences were statistically significant (see TABLE 39).

.1.3.3 Specific mutagenicity of SWSC-I

With respect to frameshift mutation in strain TA98, the mean activity of SWSC-I was found to be 1781 revertants per milligram dry condensate for the cigarette CALYPSO-1, 1827 for AREUSE-46, 1546 for -53, and 1282 for -55 (see TABLE 38 and FIGURE 15).

With respect to base-pair substitution in strain TA100, the mean activity of SWSC-I was found to be 1130 revertants per milligram dry condensate for the cigarette CALYPSO-1, 1113 for AREUSE-46, 991 for -53, and 880 for -55 (see TABLE 38 and FIGURE 15).

The specific mutagenicity of the SWSC-I of the test cigarettes AREUSE-53 and -55 was lower than that of the reference cigarette AREUSE-46 with respect to both types of mutation (see TABLE 39). The differences were statistically significant with 1 exception (AREUSE-53, TA100). SWSC-I of CALYPSO-1 was more mutagenic than that of AREUSE-53 and -55 with respect to both types of mutation, a statistical significance being observed for AREUSE-55 (see TABLE 39).

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7.1.3.4 Mutagenicity per milligram "new tar"

... the mutagenicity was calculated on a per milligram "new tar" ...is, i.e., dry condensate yield minus nicotine and humectant ...ields, no difference was observed for MWSC-I between the ...eference cigarette AREUSE-46 and the test cigarettes AREUSE-53 ...d -55 which both contained the modified blend but different ...ctant concentrations in the filler (see TABLES 40 and 42, ...IGURE 16). For SWSC-I, a slightly lower mutagenicity was observed ...or the test cigarettes AREUSE-53 and -55 compared to that of the ...eference cigarette AREUSE-46 with respect to strain TA98 (see ...BLES 41 and 42, FIGURE 17). However, the difference is not con-...idered to be biologically relevant. It should be noted that only ... of 4 condensate batches of each research cigarette were examined ...or the presence of humectants.

7.1.4 Comment

The modification of the original blend by replacing stems with an additional amount of flue-cured tobacco was found to slightly increase the specific mutagenicity of the MWSC-I (CALYPSO-1 vs AREUSE-46).

Increasing triethylene glycol from 0 to 1 percent and glycerol from 3 to 8 percent in the modified blend was found to decrease the mutagenicity on a dry condensate basis (AREUSE-46 vs -53 and -55). However, when the mutagenicity was calculated on a "new tar" basis, the differences between the reference and the test cigarettes were no longer seen. Therefore, the reduction in the mutagenicity on a dry condensate basis seems to be related to a dilution effect caused by the transferred humectants.

7.2 Tables and Figures

CIGARETTE	HUMECTANT CONCENTRATION IN THE FILLER (%)		
	PROPYLENE GLYCOL	GLYCEROL	TRIETHYLENE GLYCOL
CALYPSO-1	1.3	2.3	0
AREUSE-46	0.7	2.3	0
-53	1.1	2.2	1.0
-55	1.0	6.8	0

Corrected values H. Spiess 1.5 2.5; 7.5

TABLE 5 HUMECTANT CONCENTRATION IN THE FILLER

Remarks: determination performed at INBIFO

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IGA-ETTE	BATCH NO. / STAT. PARAMETER	YIELD (mg/cig.) GLYCEROL	TRIETHYLENE GLYCOL
ALYPSO-1	3200	1.5	0.0
	3218	1.3	0.0
	M	1.40	0.02
AREUSE-46	3202	1.4	0.1
	3224	1.3	0.1
	M	1.33	0.06
AREUSE-53	3208	1.3	1.0
	3226	1.3	1.0
	M	1.31	0.98
AREUSE-55	3210	3.6	0.0
	3232	3.5	0.0
	M	3.55	0.00
2R1	3216	4.1	0.1
	3234	4.2	0.1
	M	4.14	0.10

TABLE 6 HUMECTANT YIELD, MWSC-I

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...IGA-...	BATCH NO. / STAT. PARAMETER	YIELD (mg/cig.) GLYCEROL	TRIETHYLENE GLYCOL
...ALYPSO-1	3201	1.7	0.1
	3219	1.9	0.2
	M	1.82	0.11
AREUSE-46	3203	1.5	0.1
	3225	1.9	0.2
	M	1.69	0.12
AREUSE-53	3209	2.1	1.5
	3227	1.5	1.0
	M	1.79	1.24
AREUSE-55	3211	6.4	0.0
	3233	6.1	0.0
	M	6.28	0.02
2R1	3217	2.4	0.2
	3235	2.0	0.2
	M	2.21	0.16

TABLE 7 HUMECTANT YIELD, SWSC-I

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CIGARETTE	BATCH NO. / STAT. PARAMETER	YIELD (mg/cig.) CONDENSATE CRUDE	CONDENSATE DRY	NICOTINE	PUFF COUNT (a) (puff/cig.)
CALYPSO-1	3200	24.3	17.3	1.18	8.5
	3204	19.8	14.7	1.00	8.3
	3218	22.3	16.8	1.12	9.0
	3222	20.7	15.9	1.07	9.0
	M	21.79	16.17	1.095	8.7
	SE	1.00	0.58	0.037	0.1
	RSD (%)	9.2	7.2	6.8	7.9
AREUSE-46	3202	24.0	18.2	1.55	9.9
	3206	23.2	17.6	1.50	10.1
	3224	22.0	17.5	1.45	10.2
	3228	21.9	17.3	1.45	10.0
	M	22.75	17.64	1.488	10.1
	SE	0.50	0.19	0.025	0.1
	RSD (%)	4.4	2.2	3.3	7.1
AREUSE-53	3208	24.0	17.6	1.36	9.1
	3212	21.5	16.5	1.27	8.9
	3226	24.6	18.9	1.43	9.3
	3230	24.8	19.0	1.41	9.3
	M	23.75	18.02	1.369	9.1
	SE	0.75	0.59	0.034	0.1
	RSD (%)	6.3	6.5	5.0	6.3

TABLE 8 CONDENSATE AND NICOTINE YIELD AND PUFF COUNT, MWSC-I

(a) mean of each batch obtained from 10 individual values

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CIGARETTE	BATCH NO. / STAT. PARAMETER	YIELD (mg/cig.) CONDENSATE CRUDE	CONDENSATE DRY	NICOTINE	PUFF COUNT (a) (puff/cig.)
AREUSE-55	3210	23.3	17.5	1.24	9.5
	3214	24.1	17.9	1.23	9.5
	3232	23.9	17.6	1.18	9.5
	3236	22.6	17.1	1.19	9.4
	M	23.46	17.54	1.210	9.5
	SE	0.35	0.17	0.014	0.1
	RSD (%)	2.9	1.9	2.4	5.8
2R1	3216	47.6	39.6	3.04	11.8
	3220	49.4	41.2	3.17	11.6
	3234	51.6	44.5	3.09	11.7
	3238	42.8	36.6	2.82	11.8
	M	47.84	40.45	3.032	11.7
	SE	1.88	1.65	0.074	0.1
	RSD (%)	7.9	8.2	4.9	7.0

TABLE 8 (cont.) CONDENSATE AND NICOTINE YIELD AND PUFF COUNT, MWSC-I

(a) mean of each batch obtained from 10 individual values

IGA-ETTE	BATCH NO. / STAT. PARAMETER	YIELD (mg/cig.) CONDENSATE CRUDE	DRY	NICO-TINE	PUFF COUNT (a) (puff/cig.)
CALYPSO-1	3201	30.0	20.9	1.78	8.5
	3205	30.3	21.5	2.08	8.3
	3219	30.0	22.1	2.12	9.0
	3223	34.5	23.0	2.18	9.0
	M	31.22	21.88	2.041	8.7
	SE	1.11	0.44	0.091	0.1
	RSD (%)	7.1	4.0	8.9	7.9
AREUSE-46	3203	27.3	18.9	1.86	9.9
	3207	36.6	23.0	2.38	10.1
	3225	31.1	23.7	2.49	10.2
	3229	32.2	26.0	2.66	10.0
	M	31.82	22.88	2.348	10.1
	SE	1.93	1.47	0.172	0.1
	RSD (%)	12.1	12.9	14.7	7.1
AREUSE-53	3209	33.6	25.8	2.51	9.1
	3213	41.4	27.4	2.50	8.9
	3227	31.9	20.2	2.01	9.3
	3231	34.8	27.1	2.81	9.3
	M	35.43	25.13	2.456	9.1
	SE	2.07	1.69	0.166	0.1
	RSD (%)	11.7	13.5	13.5	6.3

TABLE 9 CONDENSATE AND NICOTINE YIELD AND PUFF COUNT, SWSC-I

(a) mean of each batch obtained from 10 individual values

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CIGA-RETTE	BATCH NO. / STAT. PARAMETER	YIELD (mg/cig.) CONDENSATE CRUDE	CONDENSATE DRY	NICO-TINE	PUFF COUNT (a) (puff/cig.)
AREUSE-55	3211	36.8	26.9	2.47	9.5
	3215	42.8	28.1	2.43	9.5
	3233	40.3	27.4	2.49	9.5
	3237	33.8	27.7	2.31	9.4
	M	38.43	27.50	2.423	9.5
	SE	1.98	0.25	0.041	0.1
	RSD (%)	10.3	1.8	3.3	5.8
2R1	3217	34.9	23.1	2.64	11.8
	3221	25.6	22.7	2.39	11.6
	3235	29.0	19.5	2.25	11.7
	3239	31.2	21.5	2.56	11.8
	M	30.15	21.69	2.459	11.7
	SE	1.95	0.82	0.087	0.1
	RSD (%)	12.9	7.6	7.0	7.0

TABLE 9 (cont.) CONDENSATE AND NICOTINE YIELD AND PUFF COUNT, SWSC-I

(a) mean of each batch obtained from 10 individual values

DATE OF ASSAY (Aug.89)	CIGARETTE	CONDENSATE TYPE	NUMBER OF BATCHES ANALYZED	BACTERIAL CONTAMINATION (CFU/0.15 mg dry cond.)
15, 16	CALYPSO-1, AREUSE-46, -53, -55	MWSC-I, SWSC-I	2	0

TABLE 10 BACTERIOLOGICAL EXAMINATION OF RESEARCH SUBSTANCE

PARAMETER	RESPONSE	
	RECOMMENDED	DETERMINED
histidine requirement		
growth without histidine	0	0
growth with histidine	+	+
sensitivity to		
crystal violet	+	+
ultraviolet light	+	+
ampicillin	0	0

TABLE 11 SALMONELLA TYPHIMURIUM STRAIN TA98, PHENOTYPIC CHARACTERISTICS

Remarks: dates of determinations: 9 Aug. and 20 Sep.89

DATE OF ASSAY	DETERMI-NATION	VIABILITY (CFU/plate) PLATE 1	2	3	4	MEAN	SE	RSD (%)
15-AUG-89	start	156	175	147	-	159.3	8.3	9.0
	end	174	176	103	179	158.0	18.4	23.2
16-AUG-89	start	168	180	169	164	170.3	3.4	4.0
	end	211	173	174	181	184.8	8.9	9.7

TABLE 12 SALMONELLA TYPHIMURIUM STRAIN TA98, VIABILITY

Remarks: number of viable bacteria plated in the mutagenicity assay: mean of viability x diluting factor 1E6, mean +- SE of both substudies:
1.69E8 +- 0.06E8 CFU/plate (N= 15)

DATE OF ASSAY	SUB-STANCE	PRES-ENCE OF S9	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	SE	RSD (%)
15-AUG-89	DMSO	no	13	14	14	17	14.5	0.9	11.9
		yes	29	30	30	26	28.8	0.9	6.6
	daunomycin	no	1546	1592	1524	1580	1560.5	15.6	2.0
	MMS	no	26	39	17	23	26.3	4.6	35.4
	2-AA	yes	1332	1245	1352	1450	1344.8	42.1	6.3
	2-AF	yes	327	329	324	392	343.0	16.4	9.5
16-AUG-89	DMSO	no	13	14	17	17	15.3	1.0	13.5
		yes	23	35	30	25	28.3	2.7	19.0
	daunomycin	no	1507	1503	1469	1357	1459.0	35.1	4.8
	MMS	no	23	23	17	29	23.0	2.4	21.3
	2-AA	yes	1426	1313	1337	1431	1376.8	30.3	4.4
	2-AF	yes	342	310	316	336	326.0	7.7	4.7

TABLE 13 SALMONELLA TYPHIMURIUM STRAIN TA98, SPONTANEOUS REVERSION AND RESPONSE TO DIAGNOSTIC MUTAGENS

Remarks: doses of solvent per plate (spontaneous reversion):
50 ul DMSO, doses of diagnostic mutagens per plate:
6 ug daunomycin, 650 ug MMS, 2 ug 2-AA, 2 ug 2-AF,
mean +- SE of spontaneous reversion of both substudies:
14.9 +- 0.6 rev./plate (N= 8) in the absence and
28.5 +- 1.3 rev./plate (N= 8) in the presence of S9

PARAMETER	RESPONSE	
	RECOMMENDED	DETERMINED
histidine requirement		
growth without histidine	0	0
growth with histidine	+	+
sensitivity to		
crystal violet	+	+
ultraviolet light	+	+
ampicillin	0	0

TABLE 14 SALMONELLA TYPHIMURIUM STRAIN TA100, PHENOTYPIC CHARACTERISTICS

Remarks: dates of determinations: 9 Aug. and 20 Sep.89

DATE OF ASSAY	DETERMI-NATION	VIABILITY (CFU/plate) PLATE 1	2	3	4	MEAN	SE	RSD (%)
15-AUG-89	start	111	90	116	113	107.5	5.9	11.0
	end	109	107	103	111	107.5	1.7	3.2
16-AUG-89	start	123	129	134	120	126.5	3.1	4.9
	end	113	117	117	124	117.8	2.3	3.9

TABLE 15 SALMONELLA TYPHIMURIUM STRAIN TA100, VIABILITY

Remarks: number of viable bacteria plated in the mutagenicity assay: mean of viability x diluting factor 1E6, mean +- SE of both substudies:
1.15E8 +- 0.03E8 CFU/plate (N= 16)

DATE OF ASSAY	SUB-STANCE	PRES-ENCE OF S9	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	MEAN	SE	RSD (%)
15-AUG-89	DMSO	no	109	120	106	87	105.5	6.9	13.0
		yes	121	123	123	90	114.3	8.1	14.2
	daunomycin	no	232	210	224	108	193.5	28.9	29.8
	MMS	no	1088	1198	1014	938	1059.5	55.4	10.5
	2-AA	yes	1675	1714	1739	1567	1673.8	37.9	4.5
	2-AF	yes	285	298	309	250	285.5	12.8	9.0
16-AUG-89	DMSO	no	104	117	109	127	114.3	5.0	8.8
		yes	131	113	123	89	114.0	9.1	16.0
	daunomycin	no	145	132	131	92	125.0	11.5	18.3
	MMS	no	1418	1490	1446	936	1322.5	129.7	19.6
	2-AA	yes	1482	1538	1514	1420	1488.5	25.6	3.4
	2-AF	yes	251	288	274	226	259.8	13.6	10.5

TABLE 16 SALMONELLA TYPHIMURIUM STRAIN TA100, SPONTANEOUS REVERSION AND RESPONSE TO DIAGNOSTIC MUTAGENS

Remarks: doses of solvent per plate (spontaneous reversion):
50 ul DMSO, doses of diagnostic mutagens per plate:
6 ug daunomycin, 650 ug MMS, 2 ug 2-AA, 2 ug 2-AF,
mean +- SE of spontaneous reversion of both substudies:
109.9 +- 4.3 rev./plate (N= 8) in the absence and
114.1 +- 5.6 rev./plate (N= 8) in the presence of S9

DATE OF MUTAGENICITY ASSAY (Aug.89)	PROTEIN CONCENTRATION (g/l) UNFIL-TERED	FILTERED	AMOUNT (mg/plate)	SPECIFIC AHM ACTIVITY (U/mg protein)	BACTERIAL CONTAMI-NATION (CFU/ml)
15	3.2	3.2	1.6	99.0	0
16	3.2	3.1	1.6	100.7	0

TABLE 17 ANALYTICAL DATA OF S9 MIXES

Remarks: S9 mixes stored at -75 degrees centigrade until determination
date of determination: 25 Oct.89
protein determination according to Lowry et al. (1951)

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3200	0.00	19	27	33	33	28.0	3.3	23.7		
		0.05	98	102	93	102	98.8	2.1	4.3		
		0.10	227	210	234	213	221.0	5.7	5.2		
		0.15	334	314	324	339	327.8	5.5	3.4	2043	0.993
	3204	0.00	37	23	31	29	30.0	2.9	19.2		
		0.05	128	102	127	92	112.3	9.0	16.1		
		0.10	253	225	269	255	250.5	9.2	7.4		
		0.15	387	370	324	359	360.0	13.3	7.4	2257	0.988
	3200	0.00	-	-	-	-	29.0	2.1	20.2		
	3204	0.05	-	-	-	-	105.5	5.0	13.4		
		0.10	-	-	-	-	235.8	7.5	9.0		
		0.15	-	-	-	-	343.9	9.0	7.4	2150	0.986
16-AUG-89	3218	0.00	25	36	32	36	32.3	2.6	16.1		
		0.05	99	105	109	103	104.0	2.1	4.0		
		0.10	203	206	235	211	213.8	7.3	6.8		
		0.15	317	351	353	363	346.0	10.0	5.8	2102	0.987
	3222	0.00	28	38	37	38	35.3	2.4	13.8		
		0.05	111	122	115	115	115.8	2.3	4.0		
		0.10	233	245	226	226	232.5	4.5	3.9		
		0.15	410	389	370	362	382.8	10.7	5.6	2318	0.988
	3218	0.00	-	-	-	-	33.8	1.7	14.6		
	3222	0.05	-	-	-	-	109.9	2.6	6.8		
		0.10	-	-	-	-	223.1	5.3	6.7		
		0.15	-	-	-	-	364.4	9.7	7.5	2210	0.984
15-AUG-89	3200	0.00	-	-	-	-	31.4	1.4	18.4		
16-AUG-89	3204	0.05	-	-	-	-	107.7	2.8	10.4		
	3218	0.10	-	-	-	-	229.4	4.7	8.2		
	3222	0.15	-	-	-	-	354.1	6.9	7.8	2180	0.985

TABLE 18 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE CALYPSO-1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.03. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3202	0.00	31	38	29	26	31.0	2.5	16.4		
		0.05	106	141	121	113	120.3	7.6	12.6		
		0.10	236	259	242	216	238.3	8.9	7.4		
		0.15	388	381	394	362	381.3	6.9	3.6	2337	0.991
	3206	0.00	36	38	30	36	35.0	1.7	9.9		
		0.05	109	104	119	131	115.8	6.0	10.3		
		0.10	258	263	256	255	258.0	1.8	1.4		
		0.15	413	453	441	421	432.0	9.1	4.2	2667	0.986
	3202	0.00	-	-	-	-	33.0	1.6	13.8		
	3206	0.05	-	-	-	-	118.0	4.5	10.9		
		0.10	-	-	-	-	248.1	5.6	6.4		
		0.15	-	-	-	-	406.6	11.0	7.6	2502	0.984
16-AUG-89	3224	0.00	40	38	28	44	37.5	3.4	18.2		
		0.05	93	98	118	117	106.5	6.4	12.1		
		0.10	223	218	247	250	234.5	8.2	7.0		
		0.15	329	398	368	362	364.3	14.1	7.8	2216	0.984
	3228	0.00	35	31	29	31	31.5	1.3	8.0		
		0.05	117	123	107	101	112.0	4.9	8.8		
		0.10	247	263	272	238	255.0	7.7	6.0		
		0.15	412	362	386	365	381.3	11.6	6.1	2385	0.990
	3224	0.00	-	-	-	-	34.5	2.0	16.6		
	3228	0.05	-	-	-	-	109.3	3.9	10.1		
		0.10	-	-	-	-	244.8	6.5	7.5		
		0.15	-	-	-	-	372.8	9.0	6.9	2300	0.986
15-AUG-89	3202	0.00	-	-	-	-	33.8	1.3	15.0		
16-AUG-89	3206	0.05	-	-	-	-	113.6	3.1	10.9		
	3224	0.10	-	-	-	-	246.4	4.2	6.8		
	3228	0.15	-	-	-	-	389.7	8.1	8.4	2401	0.983

TABLE 19 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE AREUSE-46

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.08. Deviations >0.25 are considered statistically significant.

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...E OF ...AY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
5-AUG-89	3208	0.00	24	31	32	32	29.8	1.9	13.0		
		0.05	114	115	114	110	113.3	1.1	2.0		
		0.10	219	238	232	226	228.8	4.1	3.6		
		0.15	386	363	407	361	379.3	10.9	5.7	2328	0.989
	3212	0.00	23	34	25	22	26.0	2.7	21.1		
		0.05	120	109	127	124	120.0	3.9	6.6		
		0.10	253	266	255	261	258.8	3.0	2.3		
		0.15	378	410	419	371	394.5	11.8	6.0	2489	0.993
	3208	0.00	-	-	-	-	27.9	1.7	17.3		
	3212	0.05	-	-	-	-	116.6	2.3	5.5		
		0.10	-	-	-	-	243.8	6.1	7.1		
		0.15	-	-	-	-	386.9	8.0	5.8	2408	0.990
16-AUG-89	3226	0.00	37	28	36	40	35.3	2.6	14.5		
		0.05	129	116	99	130	118.5	7.2	12.2		
		0.10	225	219	211	206	215.3	4.2	3.9		
		0.15	343	335	350	356	346.0	4.5	2.6	2058	0.992
	3230	0.00	39	30	32	39	35.0	2.3	13.4		
		0.05	118	119	110	136	120.8	5.5	9.1		
		0.10	251	252	228	248	244.8	5.6	4.6		
		0.15	411	358	419	381	392.3	14.0	7.2	2391	0.988
	3226	0.00	-	-	-	-	35.1	1.6	13.0		
	3230	0.05	-	-	-	-	119.6	4.2	10.0		
		0.10	-	-	-	-	230.0	6.5	7.9		
		0.15	-	-	-	-	369.1	11.1	8.5	2225	0.984
15-AUG-89	3208	0.00	-	-	-	-	31.5	1.5	18.7		
16-AUG-89	3212	0.05	-	-	-	-	118.1	2.4	8.0		
	3226	0.10	-	-	-	-	236.9	4.7	7.9		
	3230	0.15	-	-	-	-	378.0	7.0	7.4	2316	0.986

TABLE 20 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE AREUSE-53

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.08. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3210	0.00	25	26	36	36	30.8	3.0	19.8		
		0.05	115	111	116	119	115.3	1.7	2.9		
		0.10	225	240	259	211	233.8	10.3	8.8		
		0.15	369	331	336	349	346.3	8.5	4.9	2130	0.993
	3214	0.00	28	31	32	39	32.5	2.3	14.3		
		0.05	83	123	149	100	113.8	14.3	25.2		
		0.10	232	236	242	201	227.8	9.2	8.0		
		0.15	392	339	385	376	373.0	11.8	6.3	2271	0.982
	3210	0.00	-	-	-	-	31.6	1.8	16.1		
	3214	0.05	-	-	-	-	114.5	6.7	16.5		
		0.10	-	-	-	-	230.8	6.5	7.9		
		0.15	-	-	-	-	359.6	8.4	6.6	2200	0.986
16-AUG-89	3232	0.00	40	41	35	34	37.5	1.8	9.4		
		0.05	101	109	106	86	100.5	5.1	10.2		
		0.10	209	221	209	174	203.3	10.2	10.0		
		0.15	345	343	326	314	332.0	7.4	4.4	1973	0.983
	3236	0.00	27	32	27	38	31.0	2.6	16.9		
		0.05	99	100	116	95	102.5	4.6	9.0		
		0.10	227	232	229	210	224.5	4.9	4.4		
		0.15	363	348	336	317	341.0	9.7	5.7	2104	0.990
	3232	0.00	-	-	-	-	34.3	1.9	15.7		
	3236	0.05	-	-	-	-	101.5	3.2	9.0		
		0.10	-	-	-	-	213.9	6.6	8.7		
		0.15	-	-	-	-	336.5	5.9	5.0	2038	0.986
15-AUG-89	3210	0.00	-	-	-	-	32.9	1.3	15.9		
16-AUG-89	3214	0.05	-	-	-	-	108.0	4.0	14.6		
	3232	0.10	-	-	-	-	222.3	5.0	8.9		
	3236	0.15	-	-	-	-	348.1	5.8	6.7	2119	0.984

TABLE 21 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE AREUSE-55

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.08. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3216	0.00	32	34	40	35	35.3	1.7	9.7		
		0.06	113	132	107	110	115.5	5.6	9.8		
		0.11	223	202	195	224	211.0	7.4	7.0		
		0.17	360	351	329	341	345.3	6.7	3.9	1830	0.989
	3220	0.00	32	45	37	40	38.5	2.7	14.1		
		0.05	107	94	102	95	99.5	3.1	6.2		
		0.10	185	211	205	185	196.5	6.8	6.9		
		0.15	318	317	304	290	307.3	6.6	4.3	1806	0.988
	3216	0.00	-	-	-	-	36.9	1.6	12.3		
	3220	0.05	-	-	-	-	99.5	3.1	6.2		
		0.06	-	-	-	-	115.5	5.6	9.8		
		0.10	-	-	-	-	196.5	6.8	6.9		
		0.11	-	-	-	-	211.0	7.4	7.0		
		0.15	-	-	-	-	307.3	6.6	4.3		
		0.17	-	-	-	-	345.3	6.7	3.9	1818	0.989
16-AUG-89	3234	0.00	25	31	30	23	27.3	1.9	14.2		
		0.05	92	93	101	94	95.0	2.0	4.3		
		0.10	164	171	168	165	167.0	1.6	1.9		
		0.15	279	278	258	264	269.8	5.2	3.9	1599	0.993
	3238	0.00	25	34	28	36	30.8	2.6	16.7		
		0.05	110	108	113	102	108.3	2.3	4.3		
		0.10	173	174	204	198	187.3	8.0	8.6		
		0.15	314	301	336	290	310.3	9.9	6.4	1835	0.987
	3234	0.00	-	-	-	-	29.0	1.6	15.9		
	3238	0.05	-	-	-	-	101.6	2.9	8.0		
		0.10	-	-	-	-	177.1	5.4	8.6		
		0.15	-	-	-	-	290.0	9.2	9.0	1717	0.982
15-AUG-89	3216	0.00	-	-	-	-	32.9	1.5	18.2		
16-AUG-89	3220	0.05	-	-	-	-	100.9	2.1	7.3		
	3234	0.06	-	-	-	-	115.5	5.6	9.8		
	3238	0.10	-	-	-	-	183.6	4.9	9.3		
		0.11	-	-	-	-	211.0	7.4	7.0		
		0.15	-	-	-	-	295.8	6.8	8.0		
		0.17	-	-	-	-	345.3	6.7	3.9	1775	0.984

TABLE 22 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE 2R1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.06. Deviations >0.25 are considered statistically significant.

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3200	0.00	83	75	78	78	78.5	1.7	4.2		
		0.05	147	137	115	129	132.0	6.8	10.2		
		0.10	187	151	152	-	163.3	11.8	12.6		
		0.15	209	189	208	209	203.8	4.9	4.8	815	0.970
	3204	0.00	73	104	90	101	92.0	7.0	15.2		
		0.05	167	151	162	164	161.0	3.5	4.3		
		0.10	208	191	222	204	206.3	6.4	6.2		
		0.15	288	258	312	230	272.0	17.8	13.1	1170	0.962
	3200	0.00	-	-	-	-	85.3	4.2	13.9		
	3204	0.05	-	-	-	-	146.5	6.5	12.6		
		0.10	-	-	-	-	187.9	10.3	14.6		
		0.15	-	-	-	-	237.9	15.5	18.4	999	0.905
16-AUG-89	3218	0.00	91	76	84	83	83.5	3.1	7.4		
		0.05	167	121	137	120	136.3	11.0	16.1		
		0.10	157	204	166	177	176.0	10.2	11.6		
		0.15	260	187	207	204	214.5	15.8	14.7	865	0.928
	3222	0.00	83	81	94	88	86.5	2.9	6.7		
		0.05	160	131	161	147	149.8	7.0	9.4		
		0.10	184	186	213	209	198.0	7.6	7.6		
		0.15	247	248	261	249	251.3	3.3	2.6	1085	0.986
	3218	0.00	-	-	-	-	85.0	2.0	6.8		
	3222	0.05	-	-	-	-	143.0	6.5	12.9		
		0.10	-	-	-	-	187.0	7.2	10.9		
		0.15	-	-	-	-	232.9	10.2	12.4	975	0.943
15-AUG-89	3200	0.00	-	-	-	-	85.1	2.3	10.6		
16-AUG-89	3204	0.05	-	-	-	-	144.8	4.5	12.4		
	3218	0.10	-	-	-	-	187.4	5.9	12.3		
	3222	0.15	-	-	-	-	235.4	9.0	15.3	987	0.923

TABLE 23 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE CALYPSO-1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.02. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3202	0.00	76	89	77	69	77.8	4.2	10.7		
		0.05	127	113	138	126	126.0	5.1	8.1		
		0.10	197	152	189	186	181.0	9.9	11.0		
		0.15	221	187	229	260	224.3	15.0	13.4	989	0.957
	3206	0.00	56	82	78	88	76.0	7.0	18.4		
		0.05	144	157	144	145	147.5	3.2	4.3		
		0.10	194	176	187	206	190.8	6.3	6.6		
		0.15	274	244	287	232	259.3	12.8	9.9	1186	0.975
	3202	0.00	-	-	-	-	76.9	3.8	13.9		
	3206	0.05	-	-	-	-	136.8	4.9	10.2		
		0.10	-	-	-	-	185.9	5.7	8.7		
		0.15	-	-	-	-	241.8	11.3	13.2	1087	0.955
16-AUG-89	3224	0.00	79	82	84	85	82.5	1.3	3.2		
		0.05	121	128	130	129	127.0	2.0	3.2		
		0.10	166	192	186	182	181.5	5.6	6.1		
		0.15	220	189	241	227	219.3	11.0	10.0	929	0.977
	3228	0.00	92	88	75	74	82.3	4.6	11.1		
		0.05	133	146	118	119	129.0	6.6	10.3		
		0.10	162	166	157	162	161.8	1.8	2.3		
		0.15	231	242	217	216	226.5	6.2	5.5	931	0.977
	3224	0.00	-	-	-	-	82.4	2.2	7.5		
	3228	0.05	-	-	-	-	128.0	3.2	7.1		
		0.10	-	-	-	-	171.6	4.6	7.6		
		0.15	-	-	-	-	222.9	6.0	7.6	930	0.977
15-AUG-89	3202	0.00	-	-	-	-	79.6	2.2	11.2		
16-AUG-89	3206	0.05	-	-	-	-	132.4	3.1	9.3		
	3224	0.10	-	-	-	-	178.8	4.0	9.0		
	3228	0.15	-	-	-	-	232.3	6.6	11.4	1009	0.959

TABLE 24 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE AREUSE-46

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.16. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3208	0.00	95	94	101	89	94.8	2.5	5.2		
		0.05	141	145	131	123	135.0	5.0	7.4		
		0.10	185	178	203	187	188.3	5.3	5.6		
		0.15	213	224	242	220	224.8	6.2	5.5	886	0.983
	3212	0.00	81	106	85	108	95.0	7.0	14.7		
		0.05	165	147	144	144	150.0	5.0	6.7		
		0.10	193	179	205	205	195.5	6.2	6.3		
		0.15	259	253	231	250	248.3	6.0	4.9	1010	0.982
	3208	0.00	-	-	-	-	94.9	3.4	10.2		
	3212	0.05	-	-	-	-	142.5	4.3	8.6		
		0.10	-	-	-	-	191.9	4.0	5.9		
		0.15	-	-	-	-	236.5	6.0	7.2	948	0.975
16-AUG-89	3226	0.00	91	99	87	90	91.8	2.6	5.6		
		0.05	142	149	164	143	149.5	5.1	6.8		
		0.10	214	203	187	186	197.5	6.7	6.8		
		0.15	257	253	254	209	243.3	11.4	9.4	1005	0.975
	3230	0.00	81	79	85	77	80.5	1.7	4.2		
		0.05	125	145	150	130	137.5	6.0	8.7		
		0.10	153	195	183	186	179.3	9.1	10.2		
		0.15	207	224	229	218	219.5	4.7	4.3	918	0.977
	3226	0.00	-	-	-	-	86.1	2.6	8.4		
	3230	0.05	-	-	-	-	143.5	4.3	8.4		
		0.10	-	-	-	-	188.4	6.3	9.4		
		0.15	-	-	-	-	231.4	7.3	8.9	961	0.964
15-AUG-89	3208	0.00	-	-	-	-	90.5	2.4	10.4		
16-AUG-89	3212	0.05	-	-	-	-	143.0	2.9	8.2		
	3226	0.10	-	-	-	-	190.1	3.6	7.6		
	3230	0.15	-	-	-	-	233.9	4.6	7.9	955	0.969

TABLE 25 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE AREUSE-53

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.01. Deviations >0.25 are considered statistically significant.

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2026023558

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3210	0.00	63	89	75	107	83.5	9.5	22.7		
		0.05	162	145	156	137	150.0	5.6	7.4		
		0.10	177	166	195	188	181.5	6.4	7.0		
		0.15	233	237	237	241	237.0	1.6	1.4	984	0.972
	3214	0.00	102	73	93	93	90.3	6.1	13.6		
		0.05	160	138	136	143	144.3	5.5	7.6		
		0.10	188	139	216	207	187.5	17.2	18.3		
		0.15	237	226	231	214	227.0	4.9	4.3	907	0.946
	3210	0.00	-	-	-	-	86.9	5.4	17.5		
	3214	0.05	-	-	-	-	147.1	3.8	7.3		
		0.10	-	-	-	-	184.5	8.6	13.1		
		Q.15	-	-	-	-	232.0	3.0	3.7	945	0.959
16-AUG-89	3232	0.00	75	80	99	76	82.5	5.6	13.6		
		0.05	102	126	146	131	126.3	9.1	14.5		
		0.10	145	215	181	173	178.5	14.4	16.1		
		0.15	198	194	208	191	197.8	3.7	3.7	796	0.930
	3236	0.00	78	88	77	85	82.0	2.7	6.5		
		0.05	151	148	108	128	133.8	10.0	14.9		
		0.10	152	184	157	164	164.3	7.0	8.6		
		0.15	208	183	203	215	202.3	6.9	6.8	782	0.957
	3232	0.00	-	-	-	-	82.3	2.9	9.9		
	3236	0.05	-	-	-	-	130.0	6.4	14.0		
		0.10	-	-	-	-	171.4	7.9	13.0		
		0.15	-	-	-	-	200.0	3.7	5.3	789	0.943
15-AUG-89	3210	0.00	-	-	-	-	84.6	3.0	14.2		
16-AUG-89	3214	0.05	-	-	-	-	138.6	4.2	12.2		
	3232	0.10	-	-	-	-	177.9	5.9	13.2		
	3236	0.15	-	-	-	-	216.0	4.7	8.8	867	0.936

TABLE 26 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE AREUSE-55

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.18. Deviations >0.25 are considered statistically significant.

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2026023559

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3216	0.00	91	71	105	93	90.0	7.0	15.7		
		0.06	150	139	141	170	150.0	7.1	9.4		
		0.11	230	205	210	216	215.3	5.4	5.0		
		0.17	274	208	262	226	242.5	15.4	12.7	930	0.947
	3220	0.00	75	93	97	86	87.8	4.8	11.0		
		0.05	143	136	132	157	142.0	5.5	7.7		
		0.10	196	165	202	189	188.0	8.1	8.6		
		0.15	237	205	240	241	230.8	8.6	7.5	950	0.974
	3216	0.00	-	-	-	-	88.9	4.0	12.7		
	3220	0.05	-	-	-	-	142.0	5.5	7.7		
		0.06	-	-	-	-	150.0	7.1	9.4		
		0.10	-	-	-	-	188.0	8.1	8.6		
		0.11	-	-	-	-	215.3	5.4	5.0		
		0.15	-	-	-	-	230.8	8.6	7.5		
		0.17	-	-	-	-	242.5	15.4	12.7	941	0.959
16-AUG-89	3234	0.00	79	101	92	79	87.8	5.4	12.3		
		0.05	135	125	140	128	132.0	3.4	5.1		
		0.10	161	173	184	169	171.8	4.8	5.6		
		0.15	224	194	202	214	208.5	6.6	6.3	804	0.980
	3238	0.00	89	92	81	81	85.8	2.8	6.6		
		0.05	153	112	122	145	133.0	9.6	14.4		
		0.10	190	180	191	153	178.5	8.9	9.9		
		0.15	213	170	199	201	195.8	9.1	9.3	751	0.933
	3234	0.00	-	-	-	-	86.8	2.8	9.2		
	3238	0.05	-	-	-	-	132.5	4.7	10.1		
		0.10	-	-	-	-	175.1	4.8	7.8		
		0.15	-	-	-	-	202.1	5.7	8.0	777	0.957
15-AUG-89	3216	0.00	-	-	-	-	87.8	2.4	10.8		
16-AUG-89	3220	0.05	-	-	-	-	135.7	3.7	9.6		
	3234	0.06	-	-	-	-	150.0	7.1	9.4		
	3238	0.10	-	-	-	-	179.4	4.4	8.5		
		0.11	-	-	-	-	215.3	5.4	5.0		
		0.15	-	-	-	-	211.7	6.1	10.0		
		0.17	-	-	-	-	242.5	15.4	12.7	870	0.945

TABLE 27 MUTAGENICITY OF MWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE 2R1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.19. Deviations >0.25 are considered statistically significant.

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3201	0.00	22	29	39	42	33.0	4.6	27.9		
		0.05	107	92	108	103	102.5	3.7	7.1		
		0.10	196	198	209	179	195.5	6.2	6.3		
		0.15	293	335	314	319	315.3	8.7	5.5	1880	0.988
	3205	0.00	29	21	28	22	25.0	2.0	16.3		
		0.05	88	106	99	100	98.3	3.8	7.6		
		0.10	176	174	184	202	184.0	6.4	6.9		
		0.15	275	266	272	267	270.0	2.1	1.6	1642	0.996
	3201	0.00	-	-	-	-	29.0	2.8	27.1		
	3205	0.05	-	-	-	-	100.4	2.6	7.2		
		0.10	-	-	-	-	189.8	4.7	6.9		
		0.15	-	-	-	-	292.6	9.5	9.2	1760	0.986
16-AUG-89	3219	0.00	33	28	36	30	31.8	1.8	11.0		
		0.05	82	108	103	93	96.5	5.8	11.9		
		0.10	170	226	191	200	196.8	11.6	11.8		
		0.15	316	328	321	334	324.8	3.9	2.4	1958	0.984
	3223	0.00	42	38	38	38	39.0	1.0	5.1		
		0.05	100	88	99	110	99.3	4.5	9.1		
		0.10	163	176	197	173	177.3	7.1	8.1		
		0.15	287	261	292	307	286.8	9.6	6.7	1643	0.984
	3219	0.00	-	-	-	-	35.4	1.7	13.3		
	3223	0.05	-	-	-	-	97.9	3.4	9.9		
		0.10	-	-	-	-	187.0	7.3	11.0		
		0.15	-	-	-	-	305.8	8.6	8.0	1800	0.979
15-AUG-89	3201	0.00	-	-	-	-	32.2	1.8	21.9		
16-AUG-89	3205	0.05	-	-	-	-	99.1	2.1	8.4		
	3219	0.10	-	-	-	-	188.4	4.2	8.9		
	3223	0.15	-	-	-	-	299.2	6.4	8.6	1780	0.982

TABLE 28 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE CALYPSO-1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.02. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3203	0.00	31	27	26	41	31.3	3.4	21.9		
		0.05	88	104	87	101	95.0	4.4	9.2		
		0.10	188	174	196	199	189.3	5.6	5.9		
		0.15	347	306	283	312	312.0	13.2	8.5	1873	0.982
	3207	0.00	29	25	35	35	31.0	2.4	15.8		
		0.05	88	99	110	-	99.0	6.4	11.1		
		0.10	183	160	182	185	177.5	5.9	6.6		
		0.15	296	297	280	-	291.0	5.5	3.3	1690	0.989
	3203	0.00	-	-	-	-	31.1	1.9	17.7		
	3207	0.05	-	-	-	-	96.7	3.4	9.4		
		0.10	-	-	-	-	183.4	4.4	6.7		
		0.15	-	-	-	-	303.0	8.5	7.4	1790	0.983
16-AUG-89	3225	0.00	40	36	31	31	34.5	2.2	12.6		
		0.05	80	98	98	74	87.5	6.2	14.1		
		0.10	201	189	151	163	176.0	11.5	13.1		
		0.15	313	322	271	253	289.8	16.5	11.4	1708	0.970
	3229	0.00	32	40	35	32	34.8	1.9	10.9		
		0.05	115	132	123	90	115.0	9.0	15.7		
		0.10	220	216	231	220	221.8	3.2	2.9		
		0.15	311	358	333	351	338.3	10.5	6.2	2035	0.991
	3225	0.00	-	-	-	-	34.6	1.3	10.9		
	3229	0.05	-	-	-	-	101.3	7.3	20.3		
		0.10	-	-	-	-	198.9	10.3	14.6		
		0.15	-	-	-	-	314.0	12.9	11.6	1871	0.969
15-AUG-89	3203	0.00	-	-	-	-	32.9	1.2	14.9		
16-AUG-89	3207	0.05	-	-	-	-	99.1	4.1	16.0		
	3225	0.10	-	-	-	-	191.1	5.7	12.0		
	3229	0.15	-	-	-	-	308.9	7.8	9.8	1833	0.974

TABLE 29 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE AREUSE-46

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.04. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3209	0.00	24	28	31	27	27.5	1.4	10.5		
		0.05	84	96	82	88	87.5	3.1	7.1		
		0.10	184	164	145	181	168.5	9.0	10.7		
		0.15	294	286	252	302	283.5	11.0	7.8	1698	0.981
	3213	0.00	30	30	37	31	32.0	1.7	10.5		
		0.05	84	74	94	69	80.3	5.5	13.8		
		0.10	194	166	184	159	175.8	8.0	9.2		
		0.15	243	266	266	247	255.5	6.1	4.8	1532	0.986
	3209	0.00	-	-	-	-	29.8	1.3	12.7		
	3213	0.05	-	-	-	-	83.9	3.2	10.9		
		0.10	-	-	-	-	172.1	5.7	9.4		
		0.15	-	-	-	-	269.5	7.9	8.3	1615	0.981
16-AUG-89	3227	0.00	37	27	52	27	35.8	5.9	33.0		
		0.05	88	77	76	89	82.5	3.5	8.4		
		0.10	136	158	141	137	143.0	5.1	7.2		
		0.15	249	247	237	228	240.3	4.9	4.0	1348	0.980
	3231	0.00	28	50	41	29	37.0	5.2	28.3		
		0.05	89	95	83	84	87.8	2.8	6.3		
		0.10	194	190	168	144	174.0	11.5	13.2		
		0.15	275	291	239	299	276.0	13.3	9.6	1607	0.975
	3227	0.00	-	-	-	-	36.4	3.7	28.5		
	3231	0.05	-	-	-	-	85.1	2.3	7.6		
		0.10	-	-	-	-	158.5	8.3	14.8		
		0.15	-	-	-	-	258.1	9.4	10.3	1477	0.967
15-AUG-89	3209	0.00	-	-	-	-	33.1	2.1	25.0		
16-AUG-89	3213	0.05	-	-	-	-	84.5	1.9	9.1		
	3227	0.10	-	-	-	-	165.3	5.2	12.5		
	3231	0.15	-	-	-	-	263.8	6.1	9.3	1546	0.974

TABLE 30 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE AREUSE-53

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.09. Deviations >0.25 are considered statistically significant.

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2026023563

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3211	0.00	42	27	28	35	33.0	3.5	21.1		
		0.05	70	73	80	88	77.8	4.0	10.3		
		0.10	135	-	125	157	139.0	9.5	11.8		
		0.15	202	187	235	267	222.8	17.8	16.0	1267	0.964
	3215	0.00	36	40	44	33	38.3	2.4	12.5		
		0.05	90	82	67	69	77.0	5.5	14.2		
		0.10	153	125	134	145	139.3	6.1	8.8		
		0.15	247	216	213	226	225.5	7.7	6.8	1248	0.976
	3211	0.00	-	-	-	-	35.6	2.2	17.4		
	3215	0.05	-	-	-	-	77.4	3.1	11.5		
		0.10	-	-	-	-	139.1	4.9	9.2		
		0.15	-	-	-	-	224.1	9.0	11.4	1257	0.970
16-AUG-89	3233	0.00	25	35	29	29	29.5	2.1	14.0		
		0.05	64	86	86	70	76.5	5.6	14.7		
		0.10	113	128	126	160	131.8	10.0	15.2		
		0.15	203	207	226	198	208.5	6.1	5.9	1185	0.979
	3237	0.00	53	37	28	37	38.8	5.2	26.8		
		0.05	85	71	77	81	78.5	3.0	7.6		
		0.10	146	151	-	-	148.5	-	-		
		0.15	238	258	275	231	250.5	10.0	8.0	1429	0.976
	3233	0.00	-	-	-	-	34.1	3.1	25.9		
	3237	0.05	-	-	-	-	77.5	3.0	10.8		
		0.10	-	-	-	-	137.3	7.3	13.0		
		0.15	-	-	-	-	229.5	9.6	11.8	1300	0.965
15-AUG-89	3211	0.00	-	-	-	-	34.9	1.9	21.3		
16-AUG-89	3215	0.05	-	-	-	-	77.4	2.1	10.8		
	3233	0.10	-	-	-	-	138.3	4.1	10.6		
	3237	0.15	-	-	-	-	226.8	6.4	11.3	1278	0.967

TABLE 31 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE AREUSE-55

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.03. Deviations >0.25 are considered statistically significant.

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3217	0.00	27	37	36	39	34.8	2.7	15.3		
		0.05	76	101	78	84	84.8	5.7	13.4		
		0.10	161	179	189	173	175.5	5.9	6.7		
		0.15	250	262	290	239	260.3	11.0	8.4	1534	0.984
	3221	0.00	34	24	28	36	30.5	2.8	18.1		
		0.05	109	104	104	101	104.5	1.7	3.2		
		0.10	217	220	203	221	215.3	4.2	3.9		
		0.15	331	305	304	310	312.5	6.3	4.0	1913	0.995
	3217	0.00	-	-	-	-	32.6	1.9	16.9		
	3221	0.05	-	-	-	-	94.6	4.6	13.8		
		0.10	-	-	-	-	195.4	8.2	11.9		
		0.15	-	-	-	-	286.4	11.5	11.3	1724	0.975
16-AUG-89	3235	0.00	27	43	25	30	31.3	4.0	25.9		
		0.05	77	82	84	78	80.3	1.7	4.1		
		0.10	178	158	145	170	162.8	7.2	8.9		
		0.15	257	214	263	255	247.3	11.2	9.1	1461	0.983
	3239	0.00	24	31	-	47	34.0	6.8	34.7		
		0.05	112	89	96	96	98.3	4.9	9.9		
		0.10	206	194	175	166	185.3	9.0	9.8		
		0.15	270	255	260	268	263.3	3.5	2.7	1559	0.991
	3235	0.00	-	-	-	-	32.4	3.4	27.8		
	3239	0.05	-	-	-	-	89.3	4.2	13.2		
		0.10	-	-	-	-	174.0	6.8	11.1		
		0.15	-	-	-	-	255.3	6.2	6.9	1513	0.982
15-AUG-89	3217	0.00	-	-	-	-	32.5	1.8	21.7		
16-AUG-89	3221	0.05	-	-	-	-	91.9	3.1	13.4		
	3235	0.10	-	-	-	-	184.7	5.9	12.7		
	3239	0.15	-	-	-	-	270.8	7.5	11.0	1619	0.973

TABLE 32 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA98, CIGARETTE 2R1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.13. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3201	0.00	84	83	75	99	85.3	5.0	11.7		
		0.05	145	147	142	164	149.5	4.9	6.6		
		0.10	219	205	210	226	215.0	4.7	4.3		
		0.15	289	277	280	263	277.3	5.4	3.9	1283	0.993
	3205	0.00	87	83	97	79	86.5	3.9	8.9		
		0.05	156	146	143	152	149.3	2.9	3.9		
		0.10	211	199	216	216	210.5	4.0	3.8		
		0.15	243	254	246	256	249.8	3.1	2.5	1102	0.990
	3201	0.00	-	-	-	-	85.9	2.9	9.7		
	3205	0.05	-	-	-	-	149.4	2.7	5.0		
		0.10	-	-	-	-	212.8	3.0	4.0		
		0.15	-	-	-	-	263.5	5.9	6.4	1192	0.987
16-AUG-89	3219	0.00	81	78	79	88	81.5	2.3	5.5		
		0.05	149	150	149	143	147.8	1.6	2.2		
		0.10	196	186	187	171	185.0	5.2	5.6		
		0.15	266	247	256	221	247.5	9.6	7.8	1070	0.982
	3223	0.00	81	81	84	95	85.3	3.3	7.8		
		0.05	150	139	151	136	144.0	3.8	5.3		
		0.10	210	181	182	194	191.8	6.8	7.1		
		0.15	245	264	235	242	246.5	6.2	5.0	1063	0.988
	3219	0.00	-	-	-	-	83.4	2.0	6.8		
	3223	0.05	-	-	-	-	145.9	2.0	4.0		
		0.10	-	-	-	-	188.4	4.1	6.2		
		0.15	-	-	-	-	247.0	5.3	6.1	1067	0.985
15-AUG-89	3201	0.00	-	-	-	-	84.6	1.7	8.2		
16-AUG-89	3205	0.05	-	-	-	-	147.6	1.7	4.6		
	3219	0.10	-	-	-	-	200.6	4.0	8.0		
	3223	0.15	-	-	-	-	255.3	4.4	6.9	1130	0.980

TABLE 33 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE CALYPSO-1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.11. Deviations >0.25 are considered statistically significant.

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0490

2026023566

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3203	0.00	75	82	90	92	84.8	3.9	9.2		
		0.05	186	150	139	144	154.8	10.7	13.8		
		0.10	211	232	219	213	218.8	4.7	4.3		
		0.15	257	235	245	258	248.8	5.5	4.4	1112	0.970
	3207	0.00	87	88	92	93	90.0	1.5	3.3		
		0.05	140	135	147	160	145.5	5.4	7.5		
		0.10	190	180	179	176	181.3	3.0	3.4		
		0.15	258	225	250	265	249.5	8.7	7.0	1028	0.981
	3203	0.00	-	-	-	-	87.4	2.2	7.0		
	3207	0.05	-	-	-	-	150.1	5.8	10.9		
		0.10	-	-	-	-	200.0	7.5	10.7		
		0.15	-	-	-	-	249.1	4.8	5.4	1070	0.971
16-AUG-89	3225	0.00	73	79	75	78	76.3	1.4	3.6		
		0.05	164	151	158	121	148.5	9.5	12.9		
		0.10	217	176	165	165	180.8	12.4	13.7		
		0.15	257	253	241	250	250.3	3.4	2.7	1108	0.967
	3229	0.00	94	89	85	89	89.3	1.8	4.1		
		0.05	173	134	155	157	154.8	8.0	10.3		
		0.10	182	196	209	222	202.3	8.6	8.5		
		0.15	315	260	266	254	273.8	14.0	10.2	1202	0.971
	3225	0.00	-	-	-	-	82.8	2.7	9.2		
	3229	0.05	-	-	-	-	151.6	5.9	11.0		
		0.10	-	-	-	-	191.5	8.1	11.9		
		0.15	-	-	-	-	262.0	8.0	8.6	1155	0.961
15-AUG-89	3203	0.00	-	-	-	-	85.1	1.8	8.3		
16-AUG-89	3207	0.05	-	-	-	-	150.9	4.0	10.6		
	3225	0.10	-	-	-	-	195.8	5.4	11.1		
	3229	0.15	-	-	-	-	255.6	4.8	7.5	1113	0.965

TABLE 34 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE AREUSE-46

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.08. Deviations >0.25 are considered statistically significant.

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0490

2026023567

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3209	0.00	92	81	108	104	96.3	6.1	12.7		
		0.05	156	152	132	151	147.8	5.4	7.3		
		0.10	204	210	206	214	208.5	2.2	2.1		
		0.15	260	258	231	236	246.3	7.4	6.0	1021	0.982
	3213	0.00	95	91	83	86	88.8	2.7	6.0		
		0.05	128	148	152	156	146.0	6.2	8.5		
		0.10	203	232	196	215	211.5	7.9	7.5		
		0.15	272	228	238	244	245.5	9.4	7.7	1072	0.973
	3209	0.00	-	-	-	-	92.5	3.4	10.4		
	3213	0.05	-	-	-	-	146.9	3.8	7.3		
		0.10	-	-	-	-	210.0	3.8	5.2		
		0.15	-	-	-	-	245.9	5.6	6.4	1046	0.977
16-AUG-89	3227	0.00	81	80	84	96	85.3	3.7	8.6		
		0.05	134	132	108	123	124.3	5.9	9.5		
		0.10	178	143	177	182	170.0	9.1	10.7		
		0.15	201	224	215	227	216.8	5.8	5.4	880	0.975
	3231	0.00	115	94	81	84	93.5	7.7	16.4		
		0.05	130	132	135	142	134.8	2.6	3.9		
		0.10	174	172	201	187	183.5	6.7	7.3		
		0.15	272	225	226	246	242.3	11.0	9.1	990	0.970
	3227	0.00	-	-	-	-	89.4	4.2	13.4		
	3231	0.05	-	-	-	-	129.5	3.6	7.9		
		0.10	-	-	-	-	176.8	5.8	9.3		
		0.15	-	-	-	-	229.5	7.5	9.3	935	0.962
15-AUG-89	3209	0.00	-	-	-	-	90.9	2.7	11.7		
16-AUG-89	3213	0.05	-	-	-	-	138.2	3.4	9.8		
	3227	0.10	-	-	-	-	193.4	5.5	11.3		
	3231	0.15	-	-	-	-	237.7	5.0	8.4	991	0.957

TABLE 35 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE AREUSE-53

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.11. Deviations >0.25 are considered statistically significant.

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DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3211	0.00	74	94	96	88	88.0	5.0	11.3		
		0.05	144	147	128	156	143.8	5.8	8.1		
		0.10	169	168	183	173	173.3	3.4	4.0		
		0.15	212	230	233	204	219.8	7.0	6.4	849	0.975
	3215	0.00	91	89	79	120	94.8	8.8	18.6		
		0.05	106	141	147	144	134.5	9.6	14.2		
		0.10	199	183	195	199	194.0	3.8	3.9		
		0.15	208	224	240	255	231.8	10.1	8.7	941	0.960
	3211	0.00	-	-	-	-	91.4	4.9	15.0		
	3215	0.05	-	-	-	-	139.1	5.5	11.1		
		0.10	-	-	-	-	183.6	4.6	7.1		
		0.15	-	-	-	-	225.8	6.1	7.7	895	0.963
16-AUG-89	3233	0.00	98	101	80	83	90.5	5.3	11.6		
		0.05	141	119	128	123	127.8	4.8	7.5		
		0.10	188	160	183	185	179.0	6.4	7.2		
		0.15	206	221	219	193	209.8	6.5	6.2	818	0.973
	3237	0.00	98	94	69	105	91.5	7.8	17.1		
		0.05	137	152	159	171	154.8	7.1	9.2		
		0.10	191	180	202	200	193.3	5.0	5.2		
		0.15	215	218	247	242	230.5	8.2	7.1	911	0.963
	3233	0.00	-	-	-	-	91.0	4.4	13.6		
	3237	0.05	-	-	-	-	141.3	6.5	12.9		
		0.10	-	-	-	-	186.1	4.6	7.0		
		0.15	-	-	-	-	220.1	6.2	8.0	864	0.954
15-AUG-89	3211	0.00	-	-	-	-	91.2	3.2	13.8		
16-AUG-89	3215	0.05	-	-	-	-	140.2	4.1	11.7		
	3233	0.10	-	-	-	-	184.9	3.2	6.8		
	3237	0.15	-	-	-	-	222.9	4.3	7.7	880	0.958

TABLE 36 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE AREUSE-55

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.03. Deviations >0.25 are considered statistically significant.

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2020023569

DATE OF ASSAY	BATCH	DOSE (mg/pl.)	MUTAGENICITY (rev./plate) PLATE 1	2	3	4	M	SE	RSD (%)	REGR. COEFF. (rev./mg)	CORR. COEFF. r
15-AUG-89	3217	0.00	102	84	90	80	89.0	4.8	10.8		
		0.05	166	165	142	140	153.3	7.1	9.2		
		0.10	185	182	217	195	194.8	7.9	8.1		
		0.15	254	219	245	237	238.8	7.4	6.2	981	0.972
	3221	0.00	87	84	67	71	77.3	4.9	12.6		
		0.05	140	169	132	167	152.0	9.4	12.4		
		0.10	206	232	220	232	222.5	6.2	5.6		
		0.15	256	271	293	265	271.3	7.9	5.8	1305	0.981
	3217	0.00	-	-	-	-	83.1	3.9	13.2		
	3221	0.05	-	-	-	-	152.6	5.5	10.1		
		0.10	-	-	-	-	208.6	7.0	9.5		
		0.15	-	-	-	-	255.0	7.9	8.8	1143	0.964
16-AUG-89	3235	0.00	85	96	90	71	85.5	5.3	12.5		
		0.05	144	147	155	123	142.3	6.8	9.6		
		0.10	196	180	176	192	186.0	4.8	5.1		
		0.15	245	234	250	224	238.3	5.8	4.9	1004	0.984
	3239	0.00	84	103	99	83	92.3	5.1	11.1		
		0.05	153	134	131	140	139.5	4.9	7.0		
		0.10	176	208	219	175	194.5	11.2	11.5		
		0.15	242	247	273	254	254.0	6.8	5.4	1080	0.977
	3235	0.00	-	-	-	-	88.9	3.7	11.6		
	3239	0.05	-	-	-	-	140.9	3.9	7.9		
		0.10	-	-	-	-	190.3	5.9	8.7		
		0.15	-	-	-	-	246.1	5.1	5.9	1042	0.978
15-AUG-89	3217	0.00	-	-	-	-	86.0	2.7	12.4		
16-AUG-89	3221	0.05	-	-	-	-	146.8	3.6	9.8		
	3235	0.10	-	-	-	-	199.4	5.0	10.0		
	3239	0.15	-	-	-	-	250.6	4.7	7.5	1093	0.967

TABLE 37 MUTAGENICITY OF SWSC-I WITH S9 ACTIVATION IN STRAIN TA100, CIGARETTE 2R1

Remarks: Difference of regression coefficients (specific mutagenicity) of both substudies relative to their mean is 0.09. Deviations >0.25 are considered statistically significant.

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TYPE OF CONDEN-SATE	MUTAGENIC EFFECT, STRAIN	CIGARETTE	SPECIFIC MUTAGENICITY (rev./mg dry cond.) N	M	SE	RSD(%)
MWSC-I	frameshift mutation, TA98	CALYPSO-1	4	2180	64	5.9
		AREUSE-46	4	2401	95	7.9
		-53	4	2317 (a)	92	8.0
		-55	4	2119	61	5.8
	base-pair substitution, TA100	CALYPSO-1	4	984 (a)	85	17.4
		AREUSE-46	4	1009	61	12.0
		-53	4	955	31	6.5
		-55	4	867	48	11.0
SWSC-I	frameshift mutation, TA98	CALYPSO-1	4	1781 (a)	82	9.2
		AREUSE-46	4	1827 (a)	81	8.8
		-53	4	1546	74	9.6
		-55	4	1282 (a)	52	8.1
	base-pair substitution, TA100	CALYPSO-1	4	1130	52	9.2
		AREUSE-46	4	1113	35	6.4
		-53	4	991	40	8.2
		-55	4	880	28	6.4

TABLE 38 SPECIFIC MUTAGENICITY OF MWSC-I AND SWSC-I

(a) The specific mutagenicity derived from the regression curve might be slightly different from the mean of 4 condensate batches calculated separately.

CONDENSATE TYPE	MUTAGENIC EFFECT, STRAIN	CIGARETTE	RELATIVE DIFFERENCE CALYPSO-1	AREUSE-46	AREUSE-53	AREUSE-55
MWSC-I	frameshift mutation, TA98	CALYPSO-1	-	-0.10=	-0.06=	0.03=
		AREUSE-46	0.10=	-	0.04=	0.12=
		-53	0.06=	-0.04=	-	0.09=
		-55	-0.03=	-0.12=	-0.09=	-
	base-pair substitution, TA100	CALYPSO-1	-	-0.03=	0.03=	0.13=
		AREUSE-46	0.03=	-	0.05=	0.15=
		-53	-0.03=	-0.05=	-	0.10=
		-55	-0.13=	-0.15=	-0.10=	-
SWSC-I	frameshift mutation, TA98	CALYPSO-1	-	-0.03=	0.14=	0.33+
		AREUSE-46	0.03=	-	0.17+	0.35+
		-53	-0.14=	-0.17+	-	0.19+
		-55	-0.33+	-0.35+	-0.19+	-
	base-pair substitution, TA100	CALYPSO-1	-	0.02=	0.13=	0.25+
		AREUSE-46	-0.02=	-	0.12=	0.23+
		-53	-0.13=	-0.12=	-	0.12=
		-55	-0.25+	-0.23+	-0.12=	-

TABLE 39 SPECIFIC MUTAGENICITY, RELATIVE DIFFERENCE

Remarks: The relative difference between the 2 research cigarettes is the difference of their mutagenicities divided by the mean of them. Negative values indicate that the mutagenicity of the cigarette given in the column is lower than that of the cigarette given in the headline.
The statistically significant difference is reached if the relative difference between 2 research cigarettes is >0.16 (absolute value, level of significance set at alpha = 0.05)

=: no statistically significant difference
+: statistically significant difference

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CIGARETTE	BATCH NO. ----- STAT. PARAMETER	"NEW TAR" YIELD (mg/cig.)	MUTAGENICITY (rev./mg "new tar") TA98	TA100
CALYPSO-1	3200	14.7	2414	963
	3218	14.3	2467	1016
	M	14.48	2441	989
AREUSE-46	3202	15.2	2797	1184
	3224	14.7	2636	1105
	M	14.94	2717	1144
AREUSE-53	3208	14.0	2924	1113
	3226	15.2	2571	1256
	M	14.60	2747	1184
AREUSE-55	3210	12.7	2932	1355
	3232	12.9	2696	1088
	M	12.81	2814	1221

TABLE 40 MUTAGENICITY CALCULATED ON A PER "NEW TAR" BASIS, MWSC-I

CIGARETTE	BATCH NO. / STAT. PARAMETER	"NEW TAR" YIELD (mg/cig.)	MUTAGENICITY (rev./mg "new tar") TA98	TA100
CALYPSO-1	3201	17.4	2265	1546
	3219	17.9	2418	1322
	M	17.63	2342	1434
AREUSE-46	3203	15.5	2290	1359
	3225	19.1	2113	1371
	M	17.30	2201	1365
AREUSE-53	3209	19.8	2218	1334
	3227	15.7	1735	1134
	M	17.72	1977	1234
AREUSE-55	3211	17.9	1895	1271
	3233	18.8	1730	1195
	M	18.36	1813	1233

TABLE 41 MUTAGENICITY CALCULATED ON A PER "NEW TAR" BASIS, SWSC-I

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CONDENSATE TYPE	MUTAGENIC EFFECT, STRAIN	CIGARETTE	RELATIVE DIFFERENCE CALYPSO-1	AREUSE-46	AREUSE-53	AREUSE-55
MWSC-I	frameshift mutation, TA98	CALYPSO-1	-	-0.11	-0.12	-0.14
		AREUSE-46	0.11	-	-0.01	-0.04
		-53	0.12	0.01	-	-0.02
		-55	0.14	0.04	0.02	-
	base-pair substitution, TA100	CALYPSO-1	-	-0.15	-0.18	-0.21
		AREUSE-46	0.15	-	-0.03	-0.07
		-53	0.18	0.03	-	-0.03
		-55	0.21	0.07	0.03	-
SWSC-I	frameshift mutation, TA98	CALYPSO-1	-	0.06	0.17	0.25
		AREUSE-46	-0.06	-	0.11	0.19
		-53	-0.17	-0.11	-	0.09
		-55	-0.25	-0.19	0.09	-
	base pair substitution, TA100	CALYPSO-1	-	0.05	0.15	0.15
		AREUSE-46	-0.05	-	0.10	0.10
		-53	-0.15	-0.10	-	0.00
		-55	-0.15	-0.10	-0.00	-

TABLE 42 MUTAGENICITY ON A PER "NEW TAR" BASIS, RELATIVE DIFFERENCE

Remarks: The relative difference between 2 research cigarettes is the difference of their mutagenicities divided by the mean of them. Negative values indicate that the mutagenicity of the cigarette given in the column is lower than that of the cigarette given in the headline.

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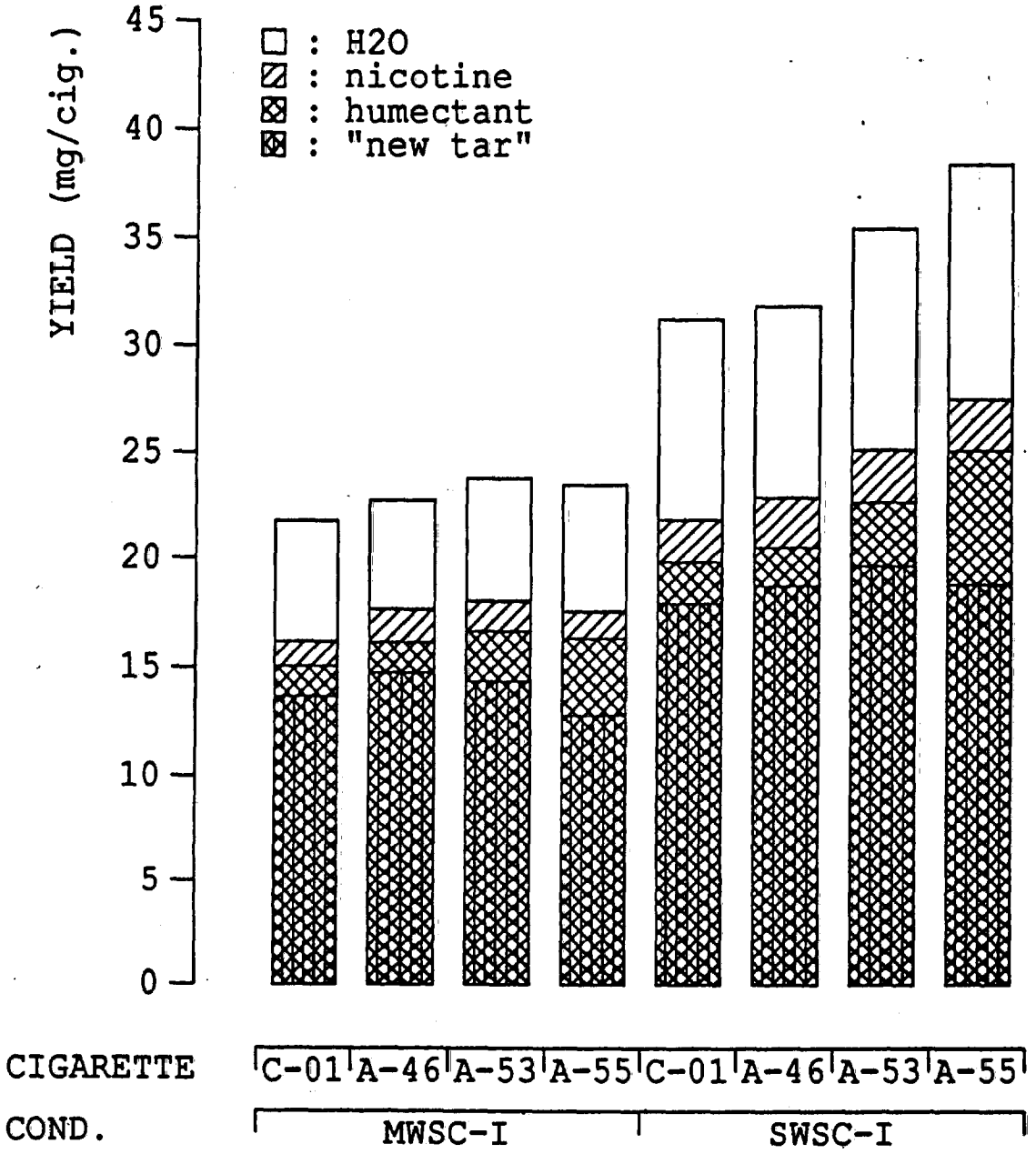

FIGURE 5 YIELD OF CRUDE CONDENSATE, DRY CONDENSATE, WATER, NICOTINE, AND HUMECTANTS (see TABLES 6 to 9)

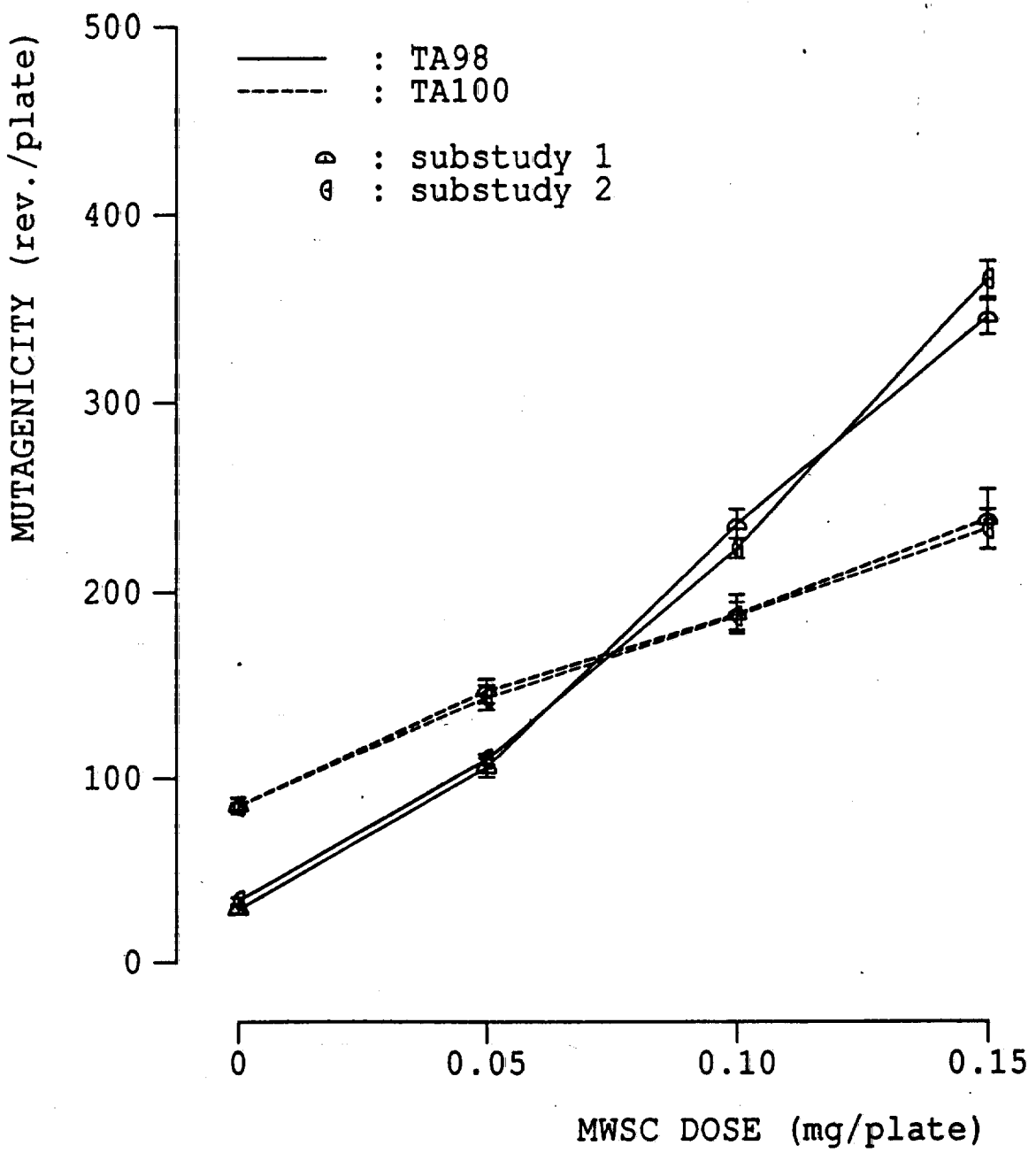

FIGURE 6 MUTAGENICITY OF MWSC-I IN STRAINS TA98 AND TA100, CIGARETTE CALYPSO-1
(see TABLES 18 and 23)

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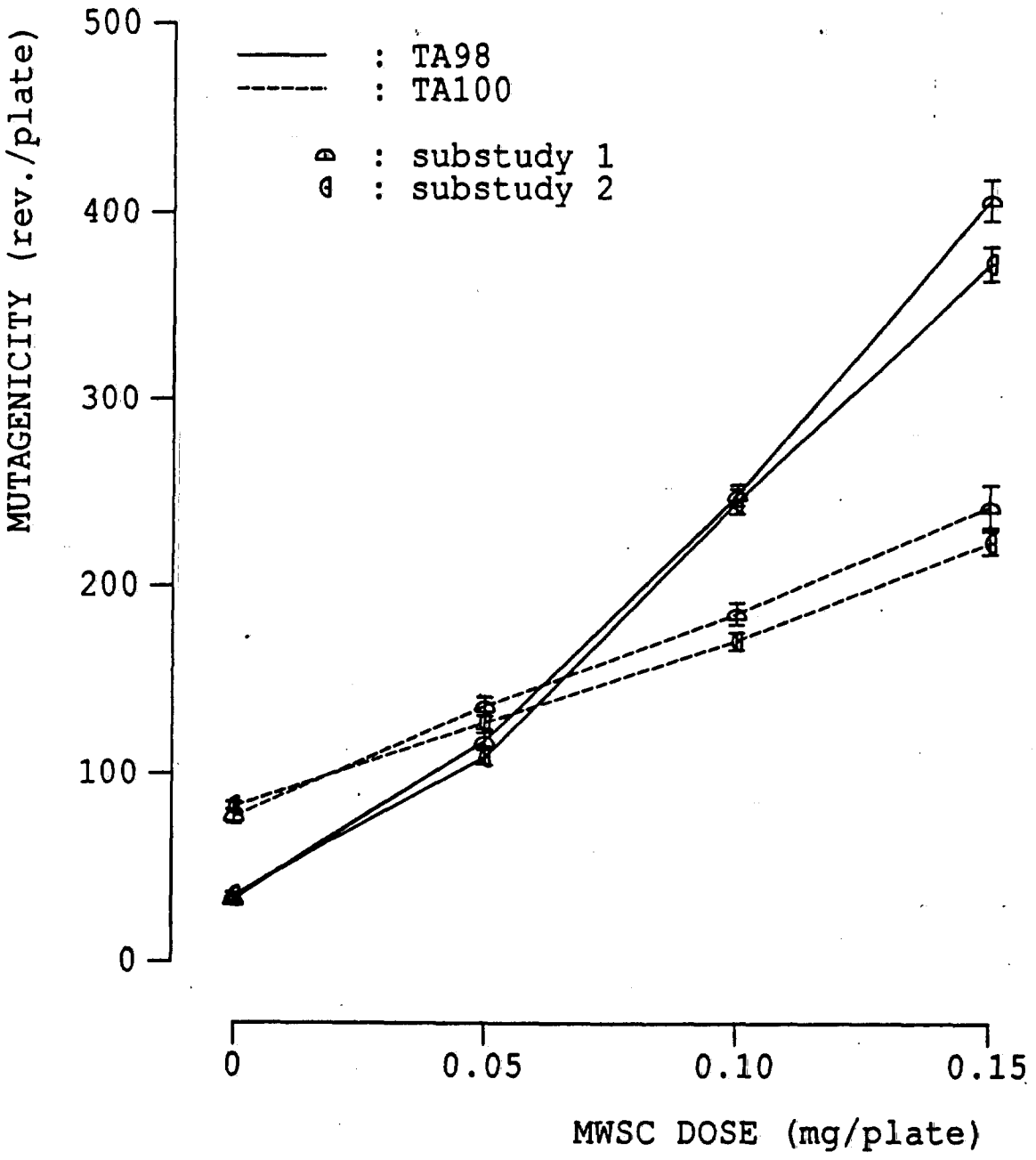

FIGURE 7 MUTAGENICITY OF MWSC-I IN STRAINS TA98 AND TA100, CIGARETTE AREUSE-46
(see TABLES 19 and 24)

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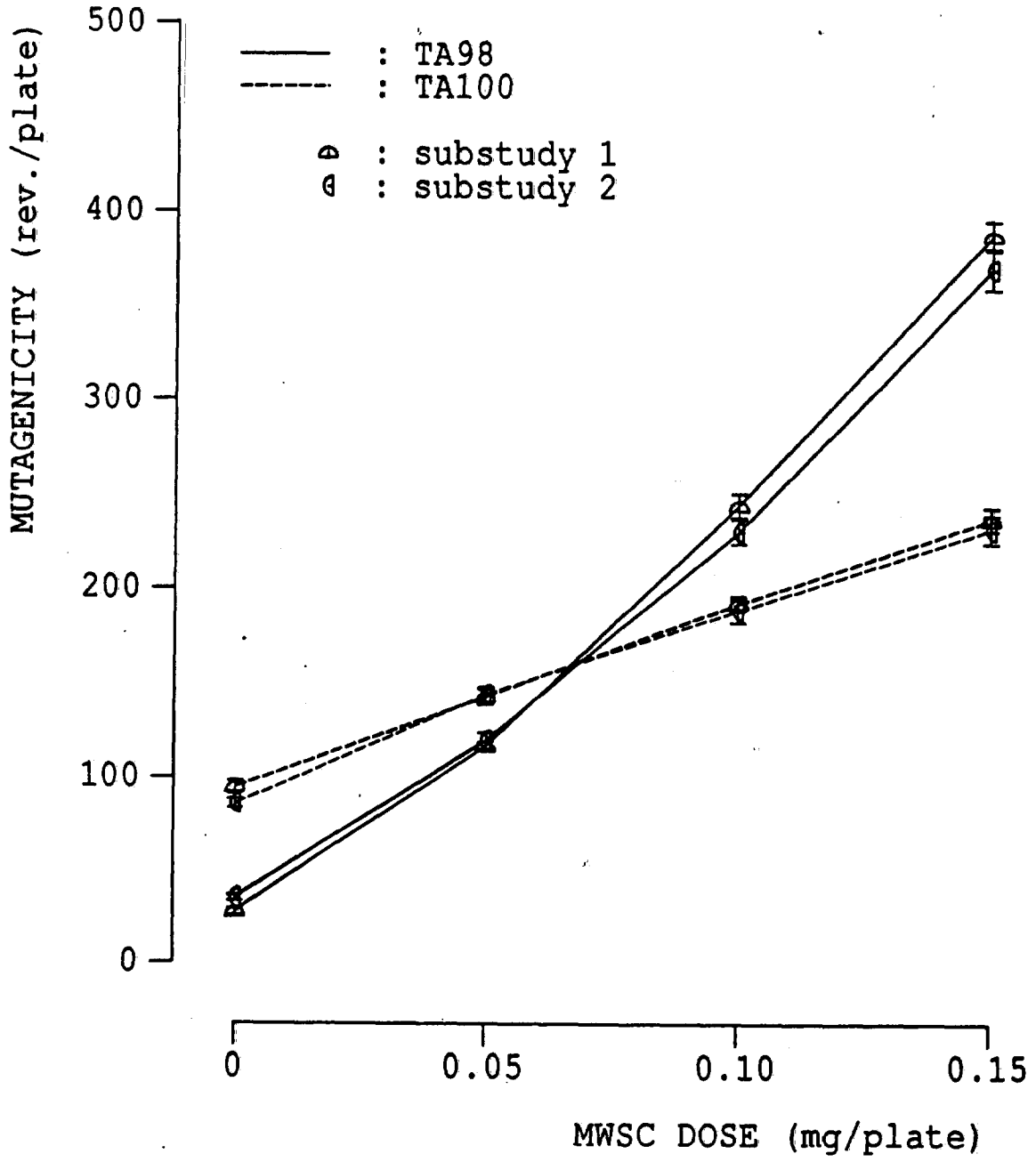

FIGURE 8 MUTAGENICITY OF MWSC-I IN STRAINS TA98 AND TA100, CIGARETTE AREUSE-53
(see TABLES 20 and 25)

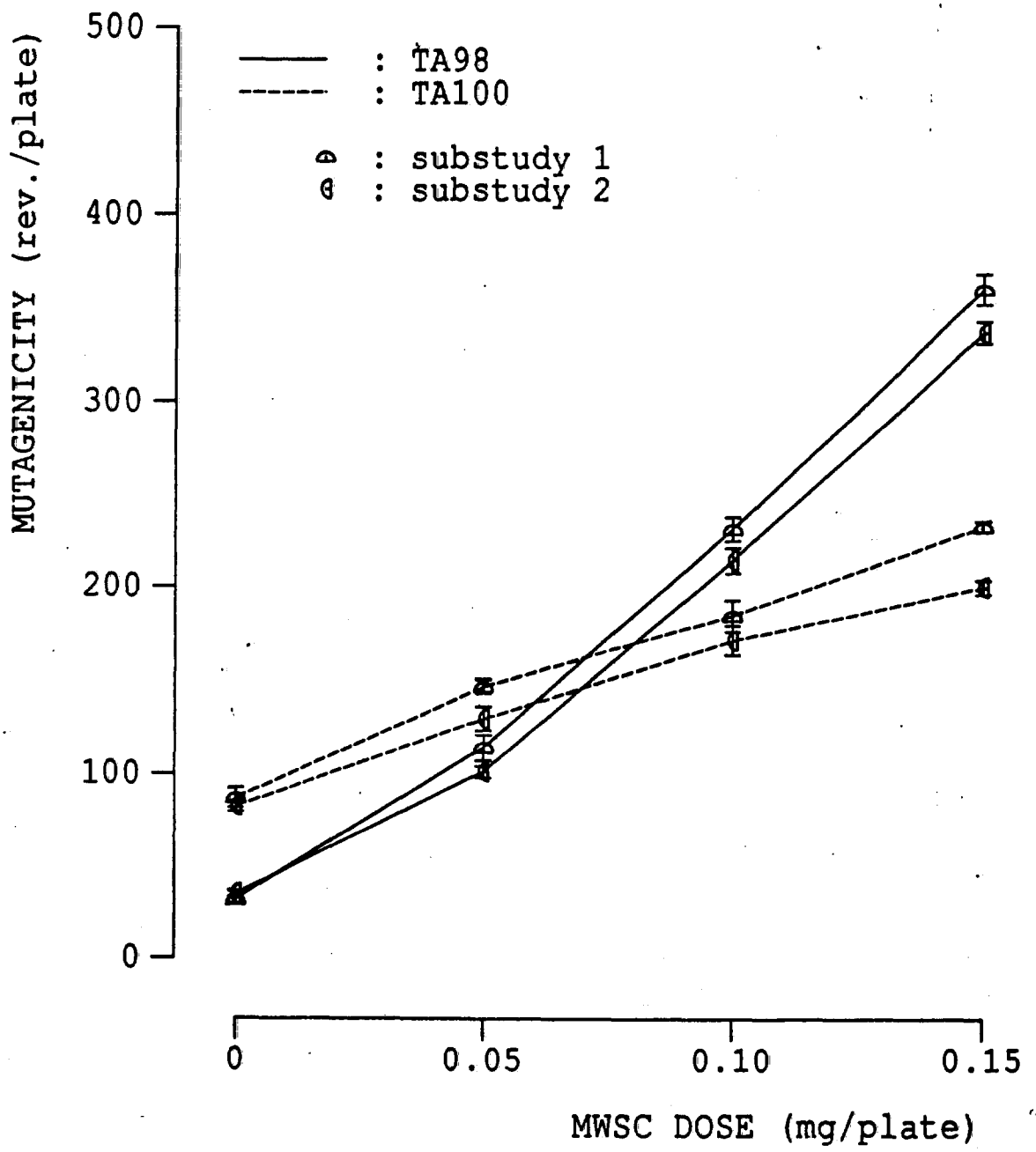

FIGURE 9 MUTAGENICITY OF MWSC-I IN STRAINS TA98 AND TA100, CIGARETTE AREUSE-55
(see TABLES 21 and 26)

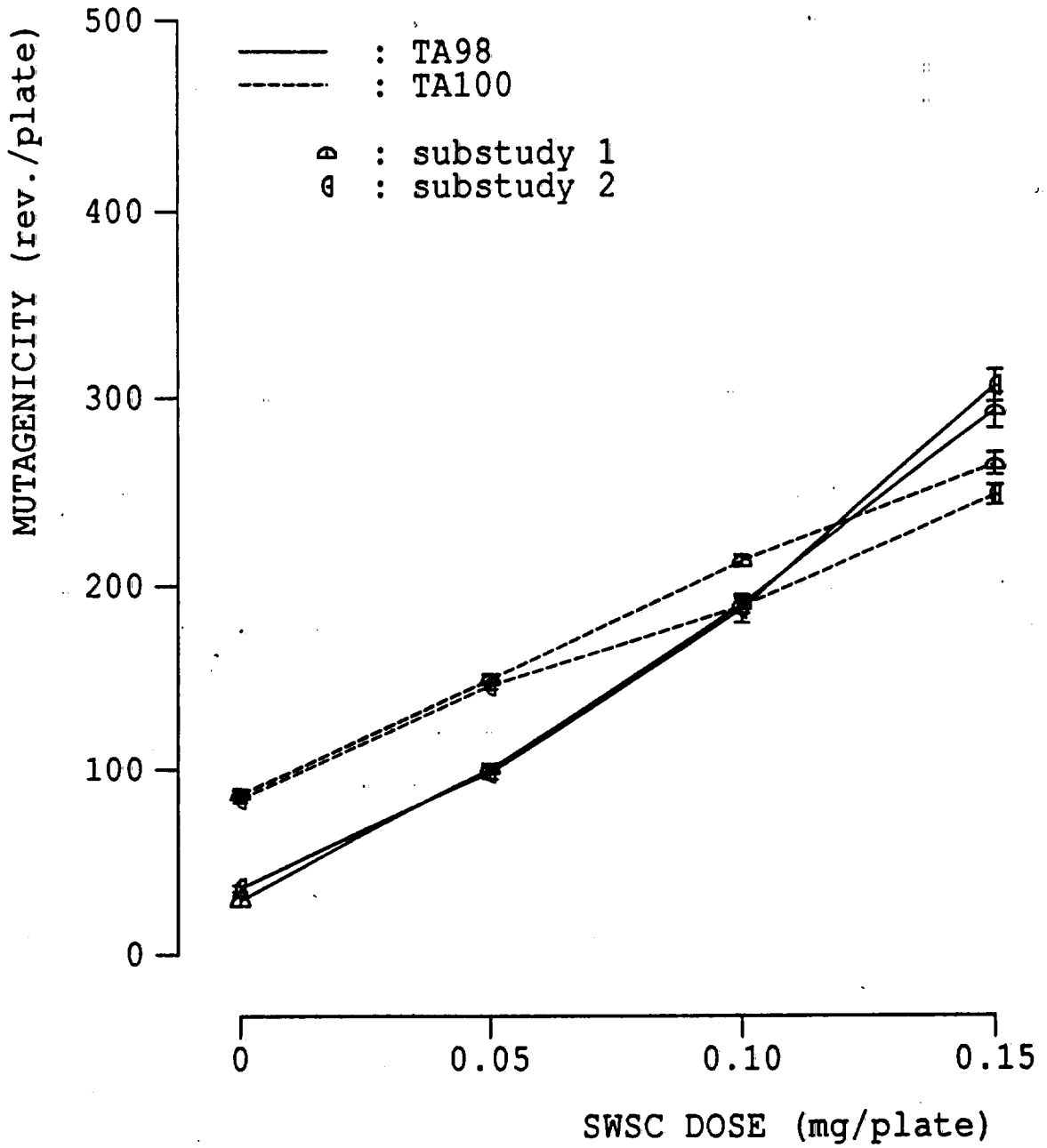

FIGURE 10 MUTAGENICITY OF SWSC-I IN STRAINS TA98 AND TA100, CIGARETTE CALYPSO-1
(see TABLES 28 and 33)

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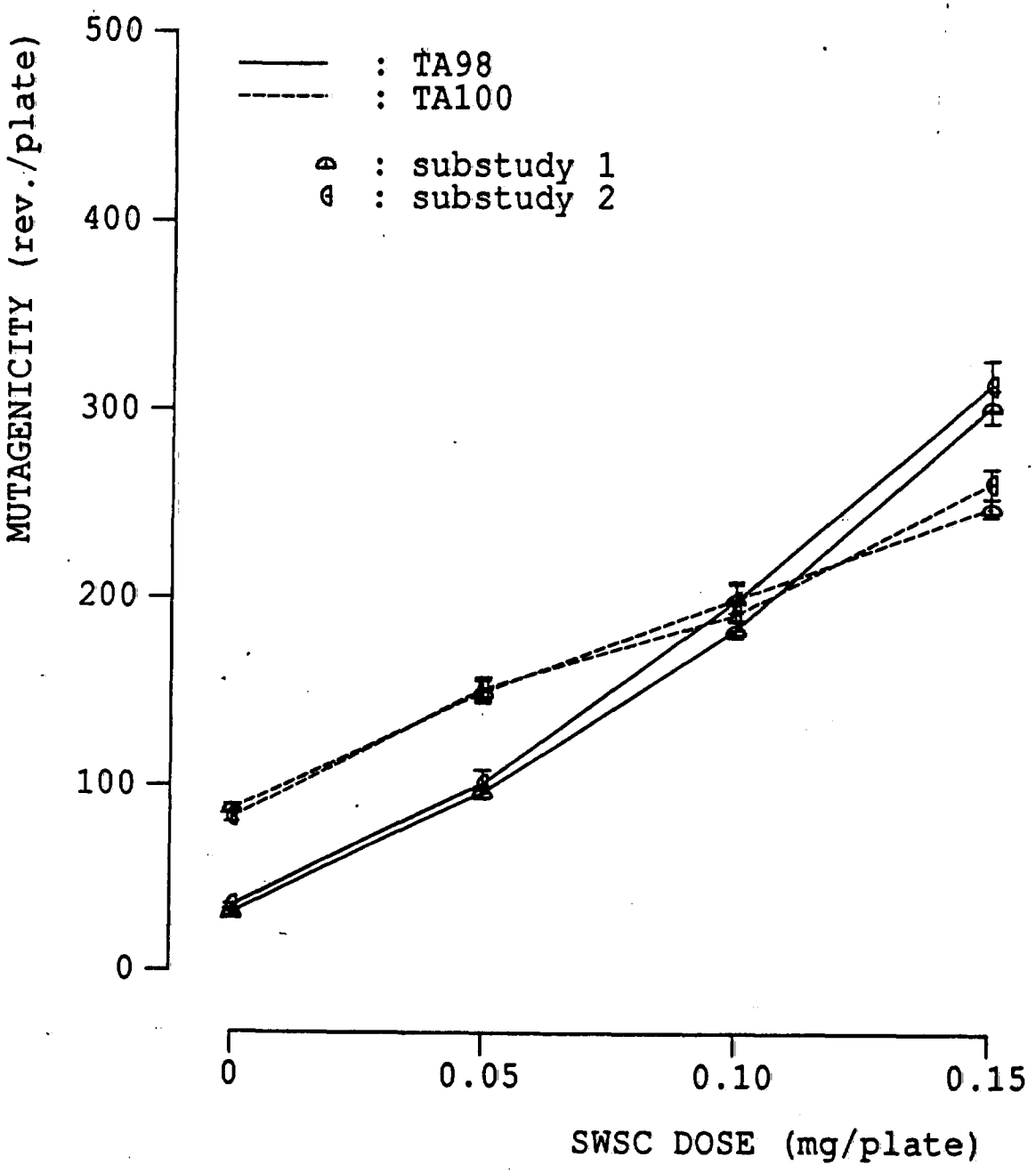

FIGURE 11 MUTAGENICITY OF SWSC-I IN STRAINS TA98 AND TA100, CIGARETTE AREUSE-46
(see TABLES 29 and 34)

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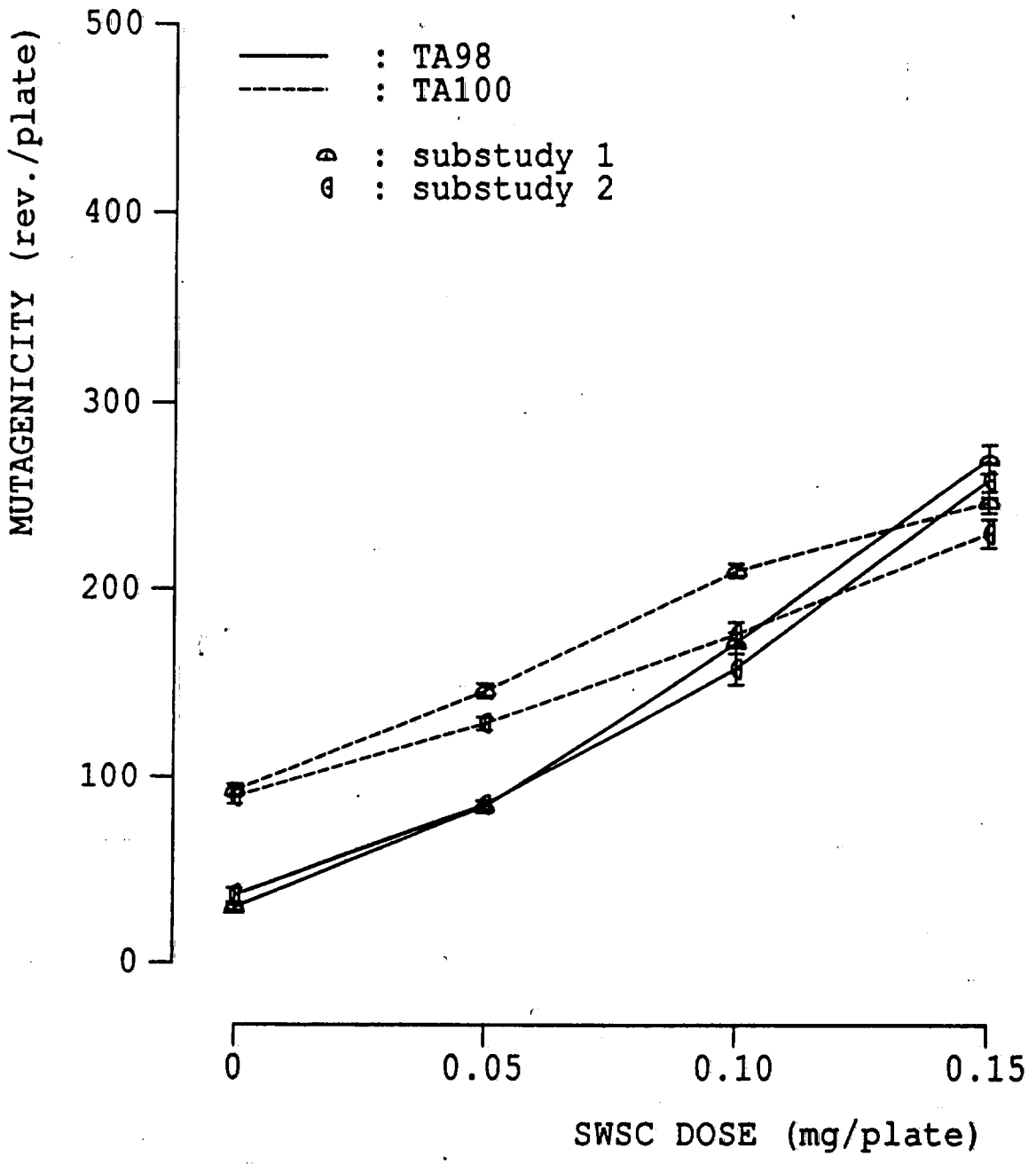

FIGURE 12 MUTAGENICITY OF SWSC-I IN STRAINS TA98 AND TA100, CIGARETTE AREUSE-53
(see TABLES 30 and 35)

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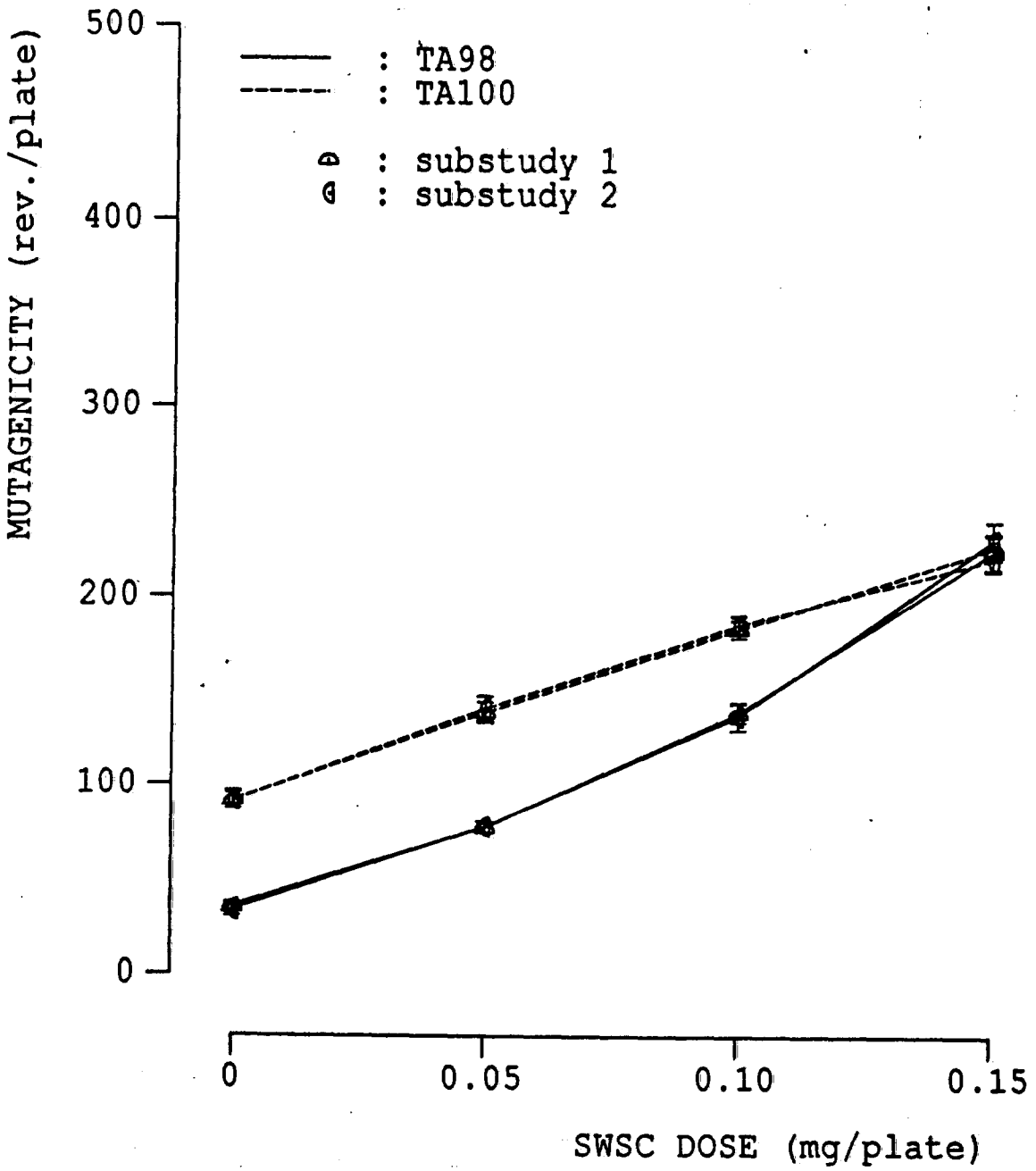

FIGURE 13 MUTAGENICITY OF SWSC-I IN STRAINS TA98 AND TA100, CIGARETTE AREUSE-55
(see TABLES 31 and 36)

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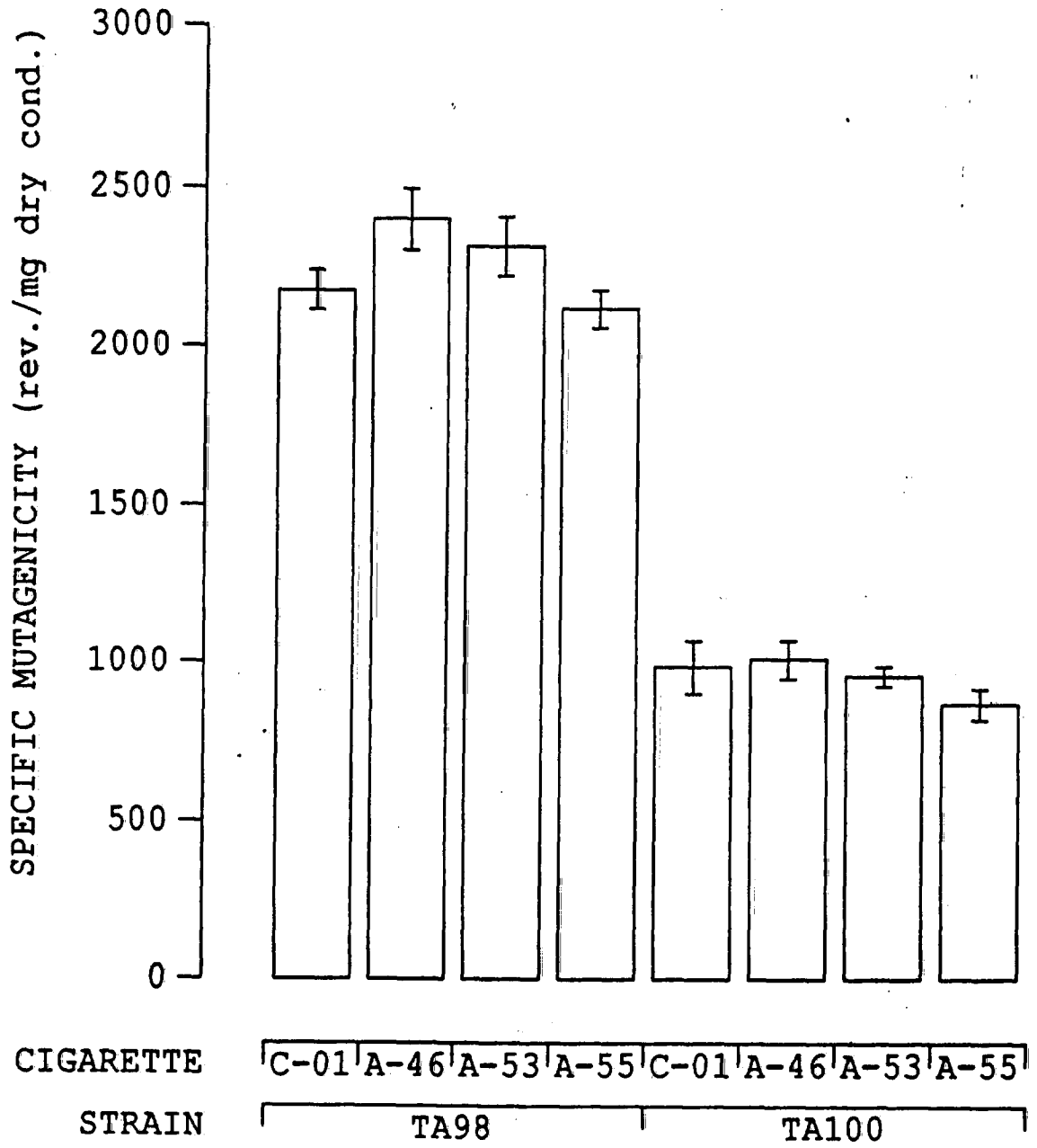

FIGURE 14 SPECIFIC MUTAGENICITY OF CIGARETTE CONDENSATES, MWSC-I (see TABLE 38)

Remarks: mean ± SE, N = 4 condensate batches

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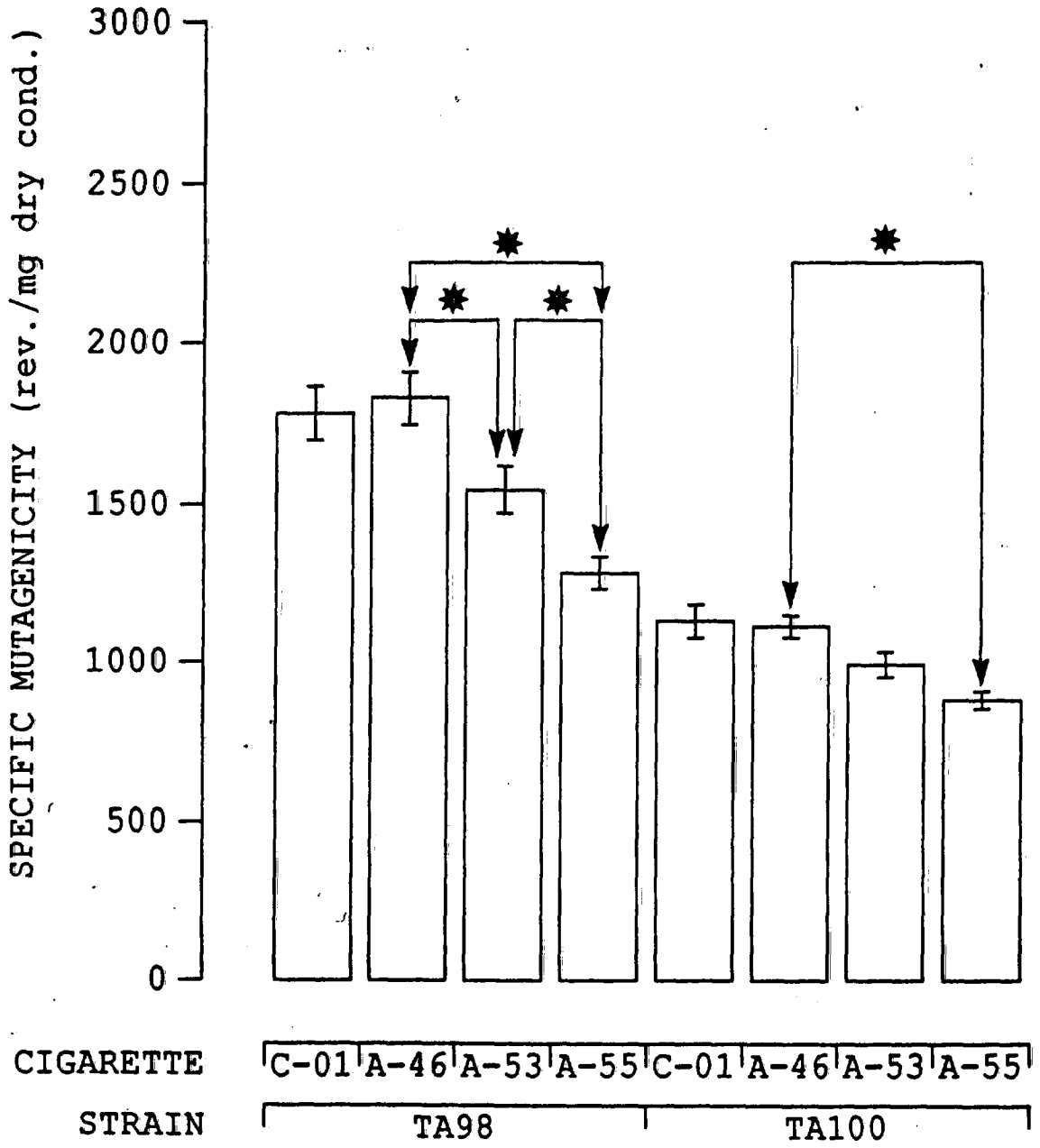

FIGURE 15 SPECIFIC MUTAGENICITY OF CIGARETTE CONDENSATES, SWSC-I (see TABLE 38)

Remarks: mean ± SE, N = 4 condensate batches
*: indicating statistically significant difference between 2 research cigarettes

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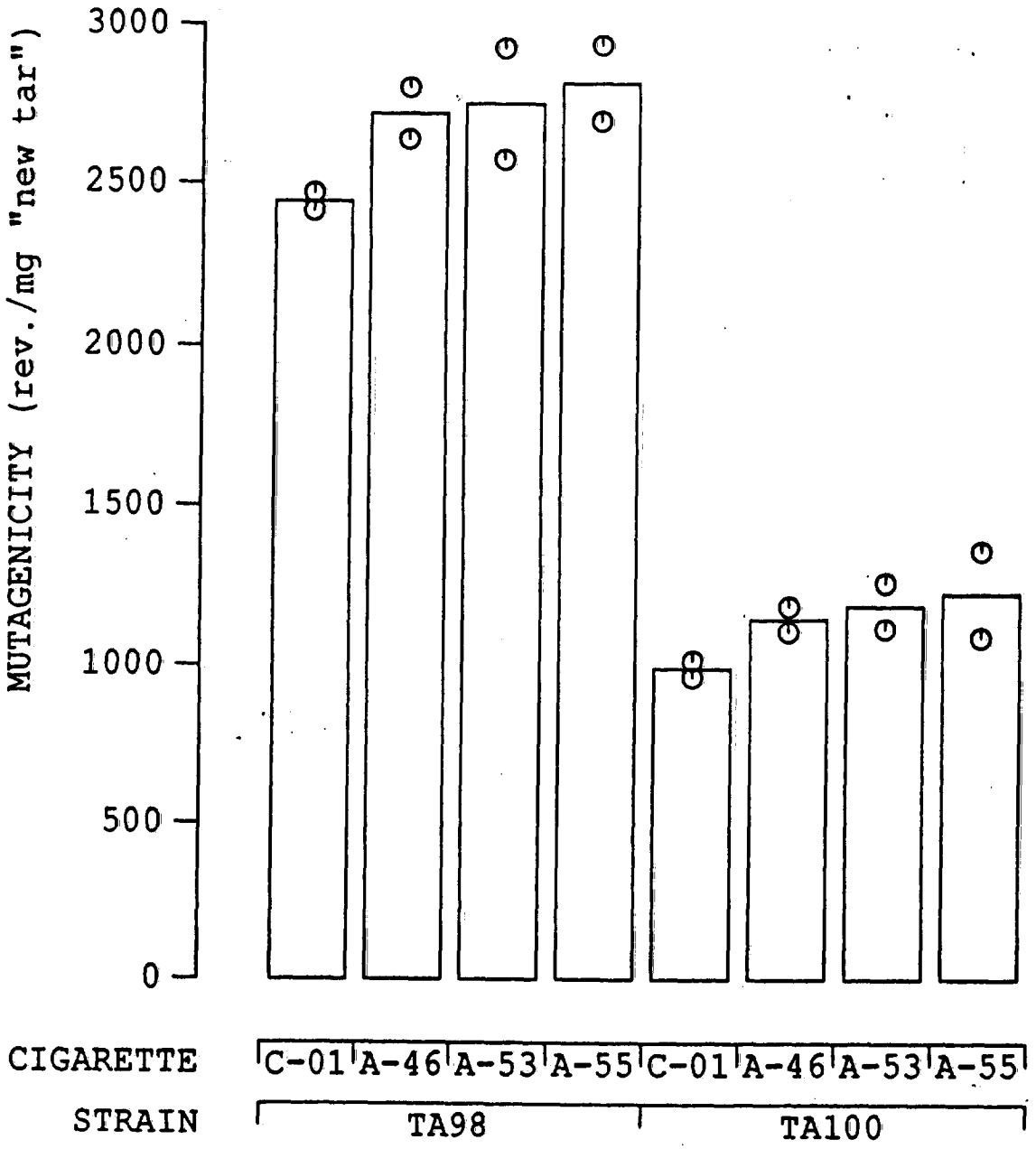

FIGURE 16 MUTAGENICITY OF CIGARETTE CONDENSATES ON A PER "NEW TAR" BASIS, MWSC-I
(see TABLE 40)

Remarks: mean and single values, N = 2 condensate batches

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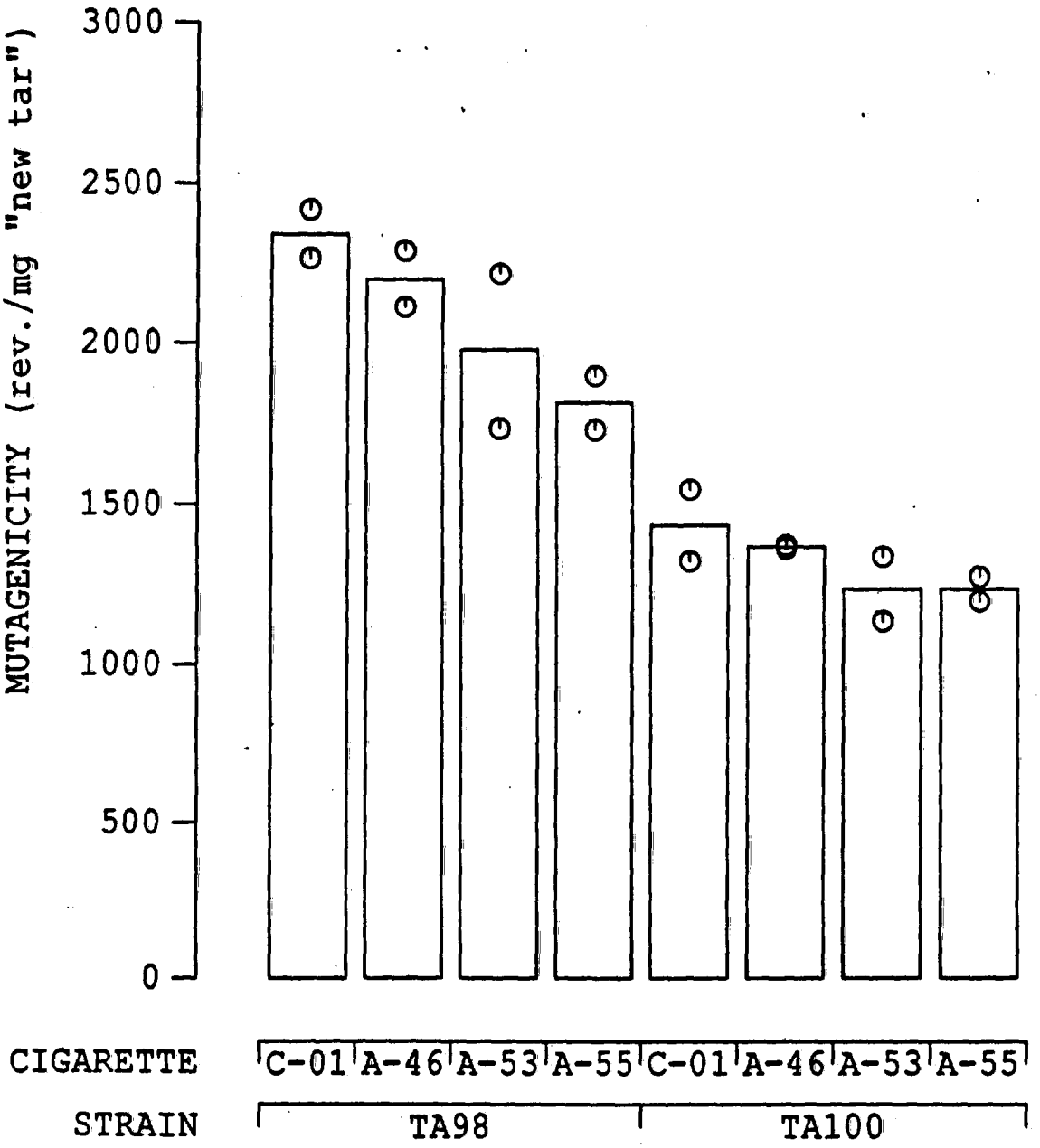

FIGURE 17 MUTAGENICITY OF CIGARETTE CONDENSATES ON A PER "NEW TAR" BASIS, SWSC-I
(see TABLE 41)

Remarks: mean and single values, N = 2 condensate batches

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END OF REPORT